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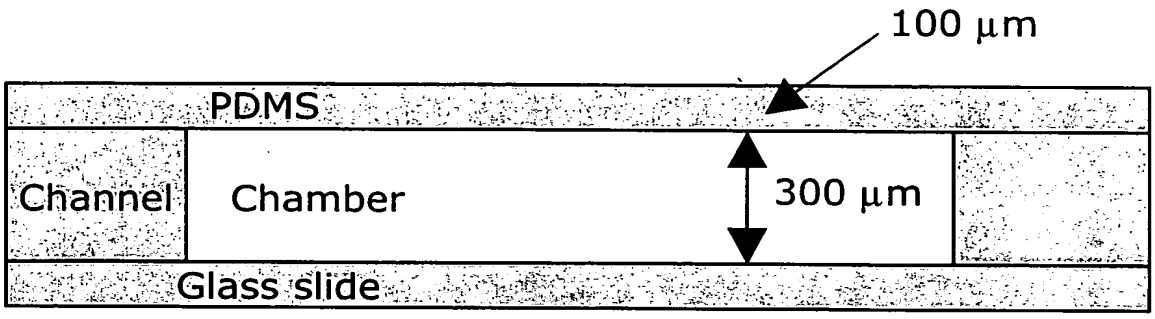


FIG. 1A

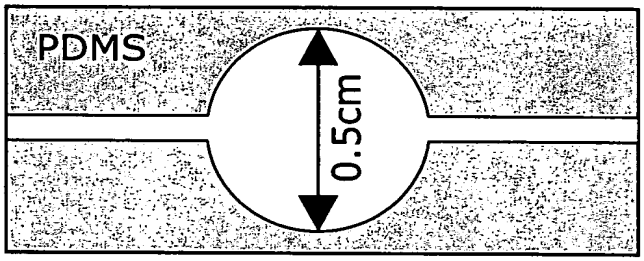


FIG. 1B

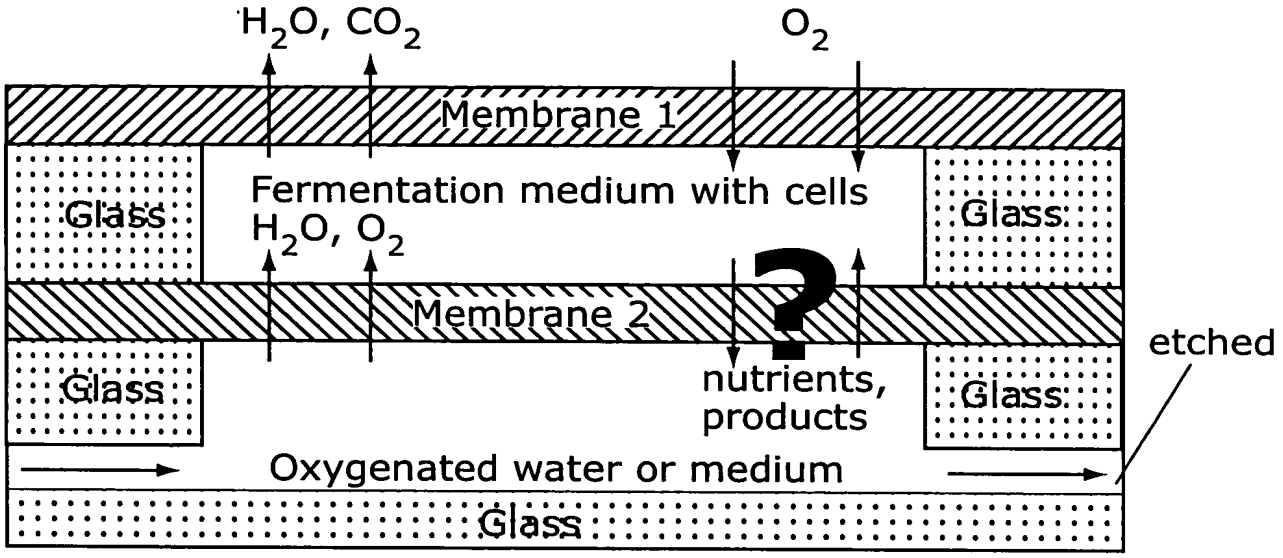

H_2O, CO_2
O_2
Membrane 1
Fermentation medium with cells
H_2O, O_2
Glass
Glass
Membrane 2
?
nutrients,
products
Glass
Glass
etched
Oxygenated water or medium
Glass

FIG. 2A

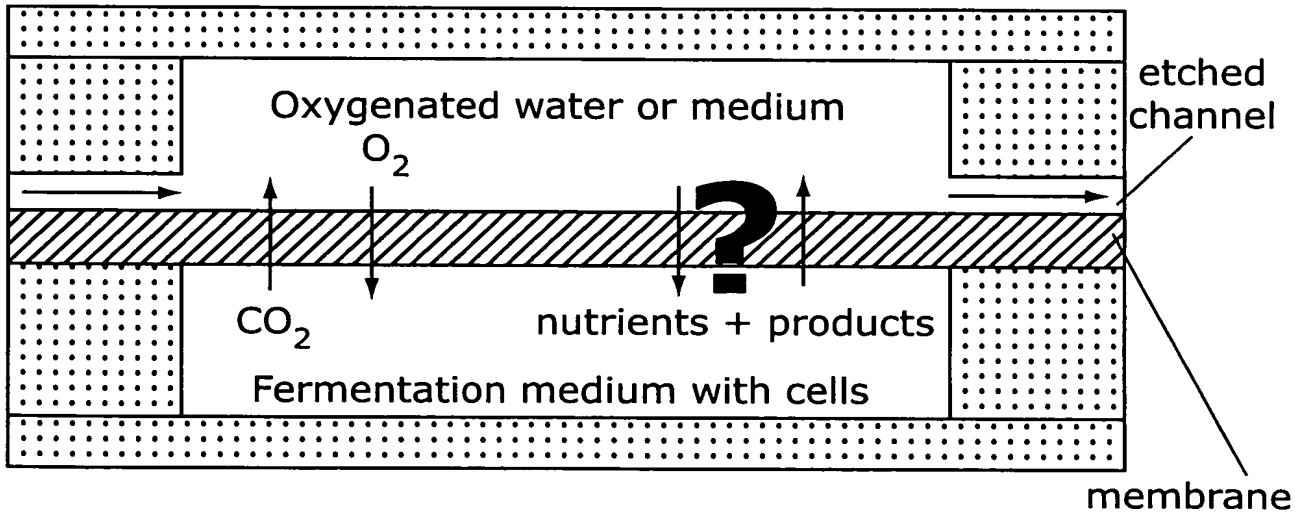

Oxygenated water or medium
O_2
?
etched
channel
CO_2
nutrients + products
Fermentation medium with cells
membrane

FIG. 2B

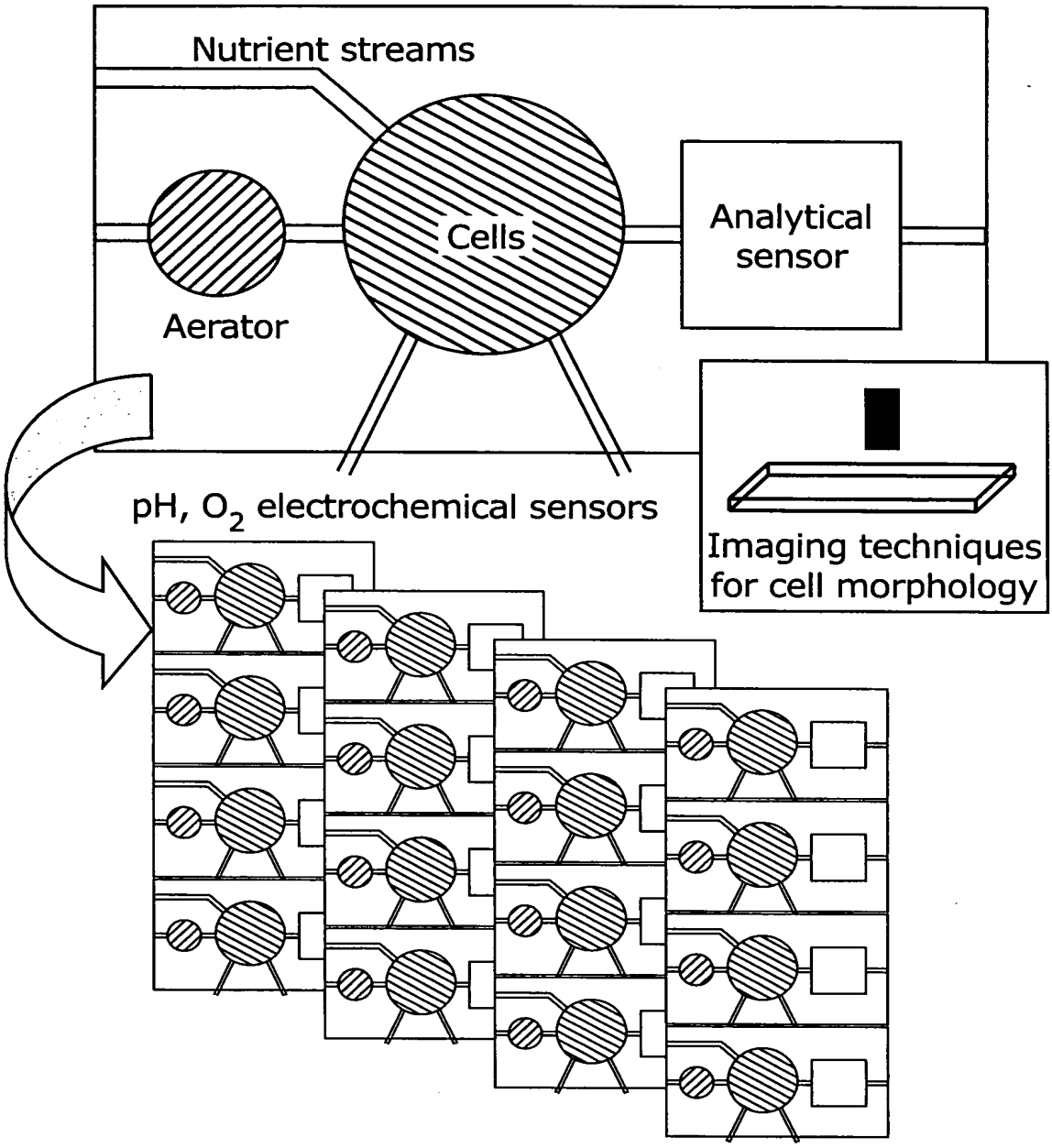

Nutrient streams
Cells
Analytical sensor
Aerator
pH, O_2 electrochemical sensors
Imaging techniques for cell morphology

FIG. 3

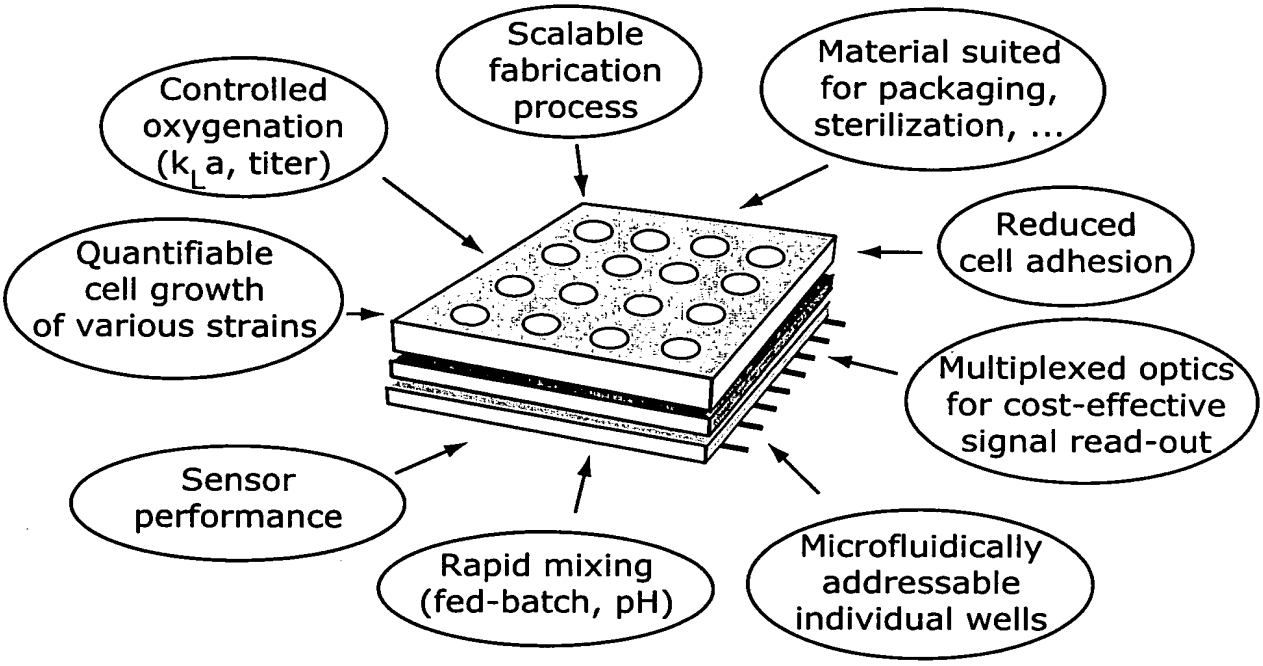

Scalable fabrication process
Material suited for packaging, sterilization, ...
Controlled oxygenation ($k_L a$, titer)
Reduced cell adhesion
Quantifiable cell growth of various strains
Multiplexed optics for cost-effective signal read-out
Sensor performance
Rapid mixing (fed-batch, pH)
Microfluidically addressable individual wells

FIG. 4A

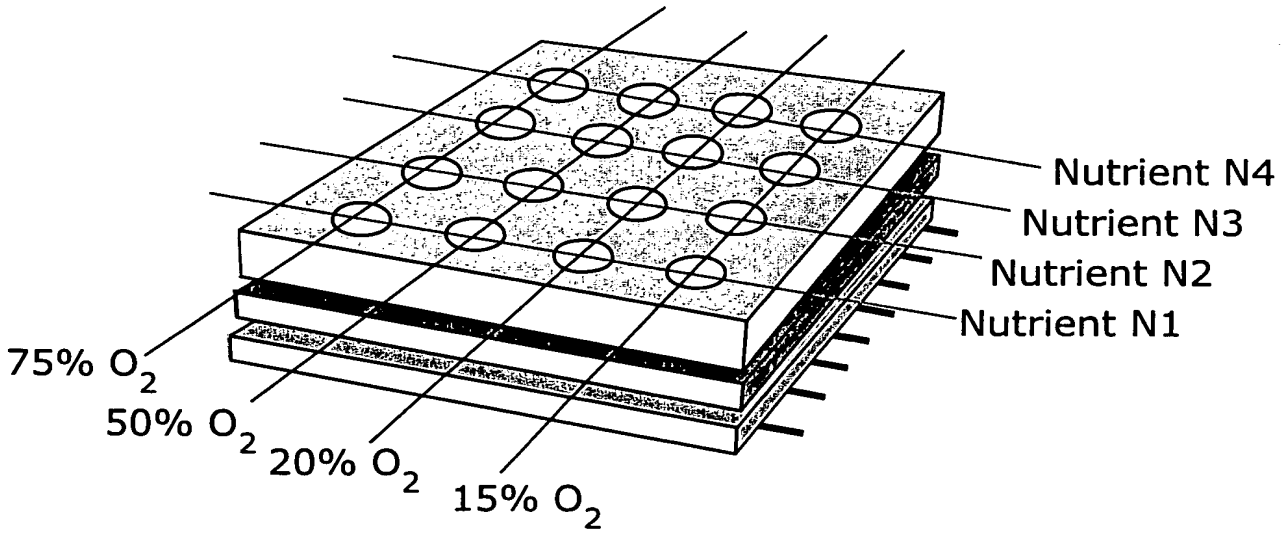

Nutrient N4
Nutrient N3
Nutrient N2
Nutrient N1
75% O_2
50% O_2
20% O_2
15% O_2

FIG. 4B

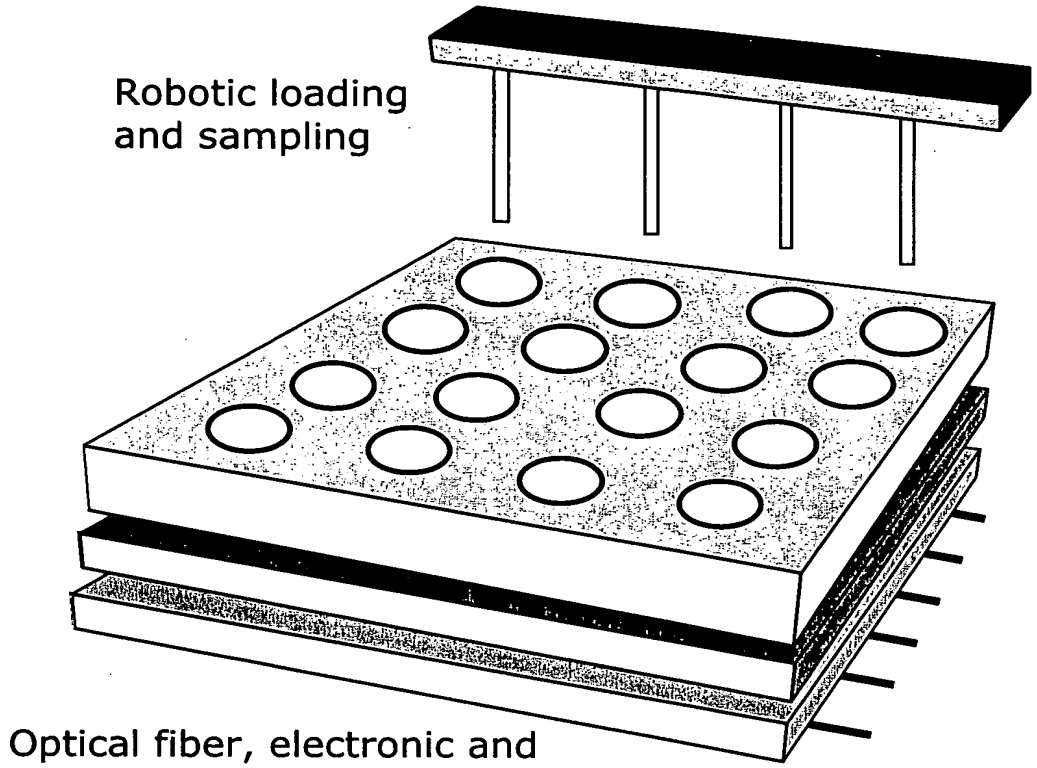
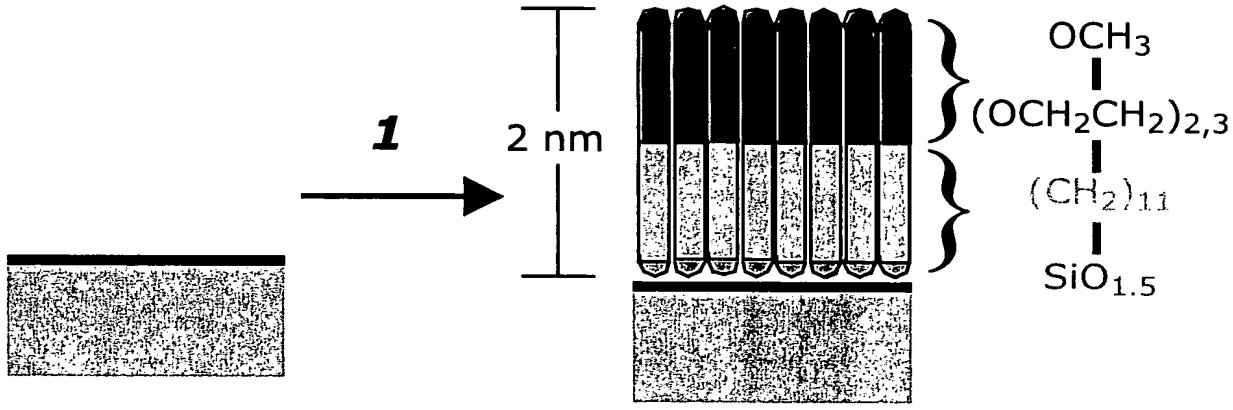


FIG. 4C



1 = $CH_3O(CH_2CH_2O)_{2,3}(CH_2)_{11}SiCl_3$

FIG. 5

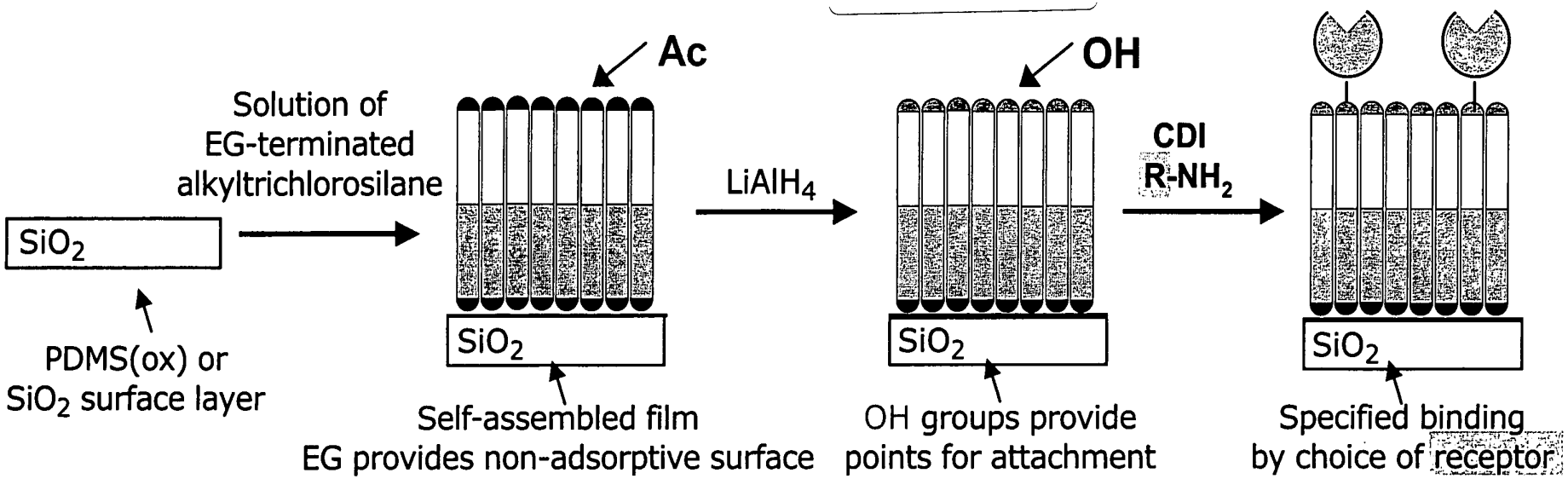
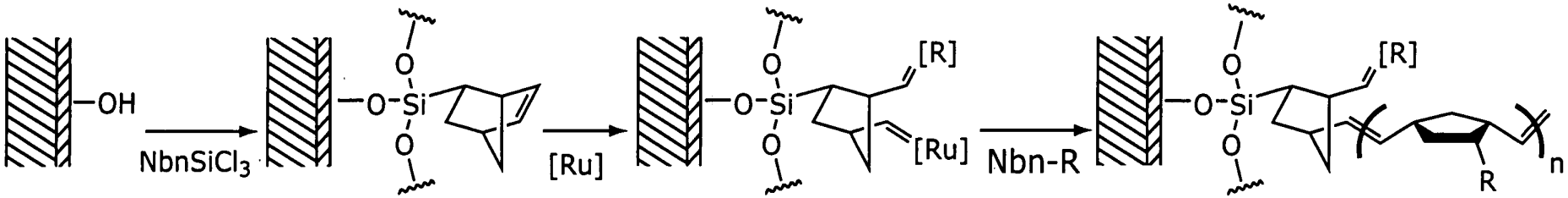
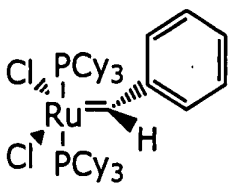


FIG. 6





Catalyst

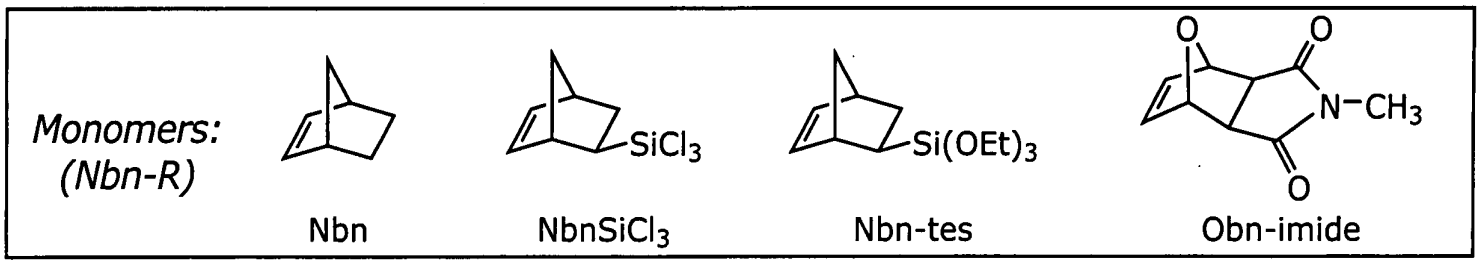


FIG. 7

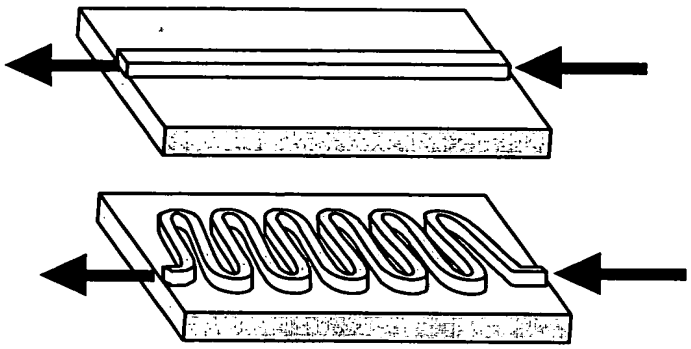


FIG. 8

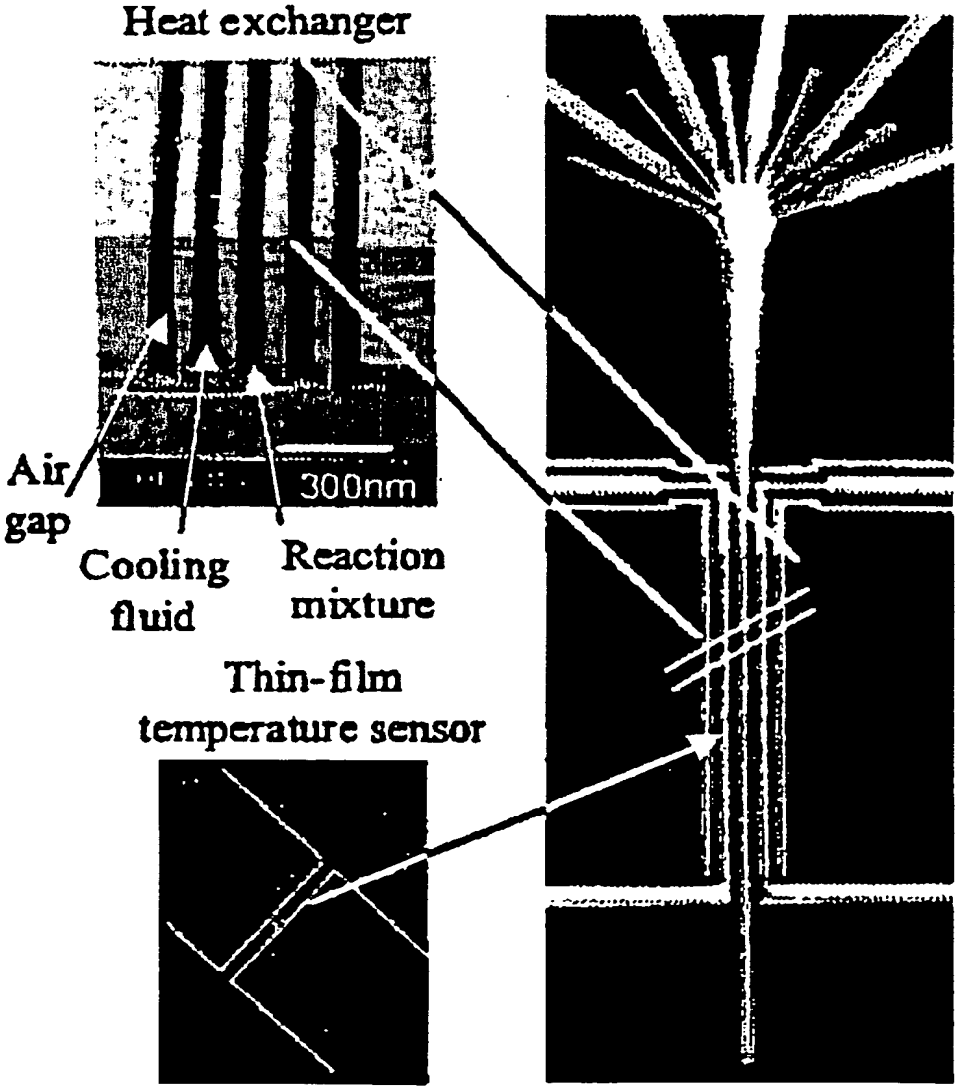
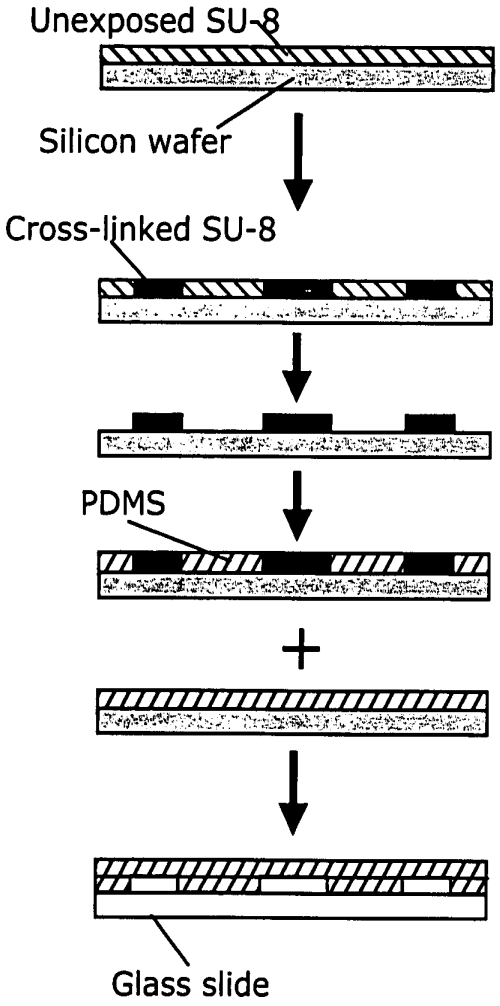

Heat exchanger
Air gap
300nm
Cooling fluid
Reaction mixture
Thin-film temperature sensor

FIG. 9

Microfermentor Fabrication

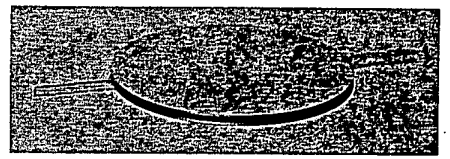


Spincoat SU-8 (photosensitive epoxy) onto wafer

Expose wafer through transparency mask

Post-bake wafer to allow SU-8 to cross-link where exposed (negative resist)

Develop wafer to create negative master mold

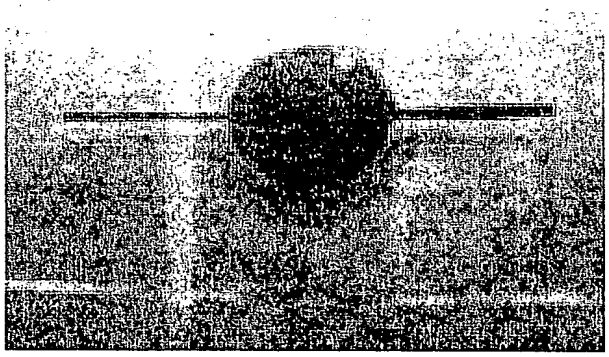


Cast fermentor walls

Spincoat PDMS onto plain wafer to create membrane

Join two layers of PDMS and bond to a glass slide (air plasma, epoxy, aquarium-grade silicone adhesive)

FIG. 10



Microfermentor filled with phenol red

FIG. 11

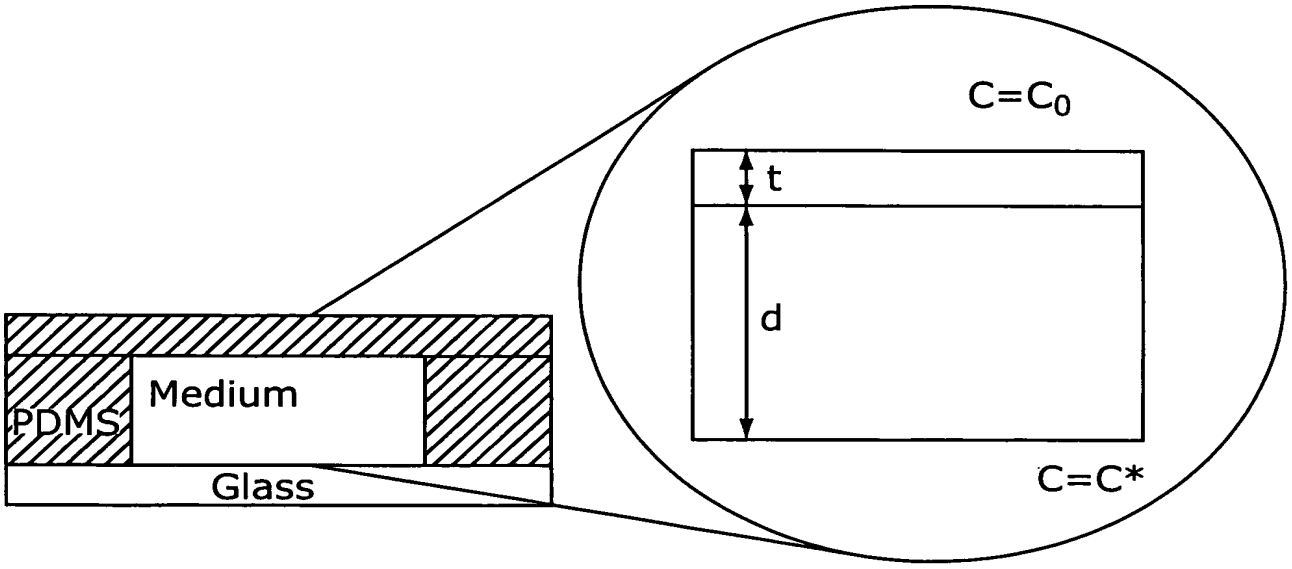


FIG. 12

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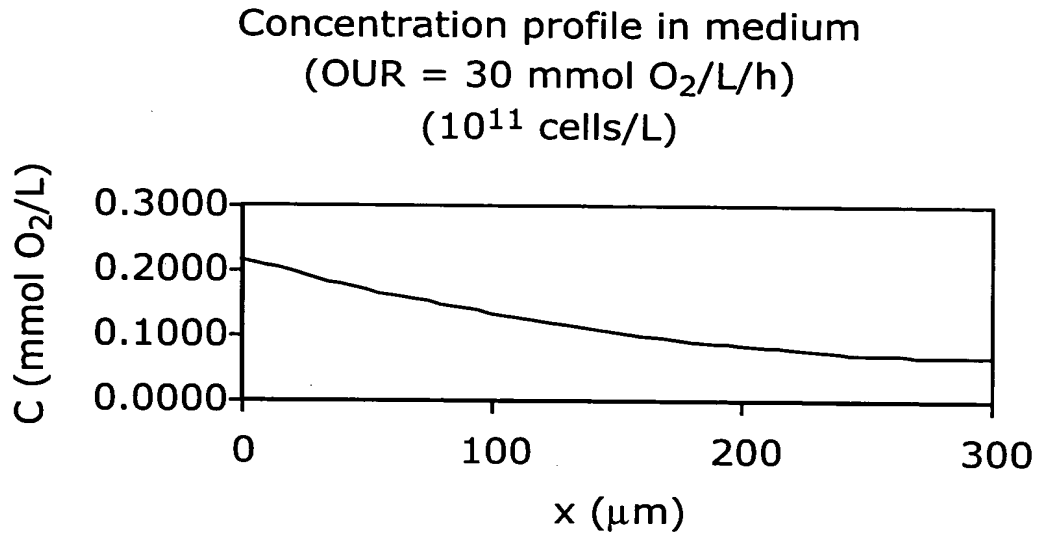


FIG. 13A

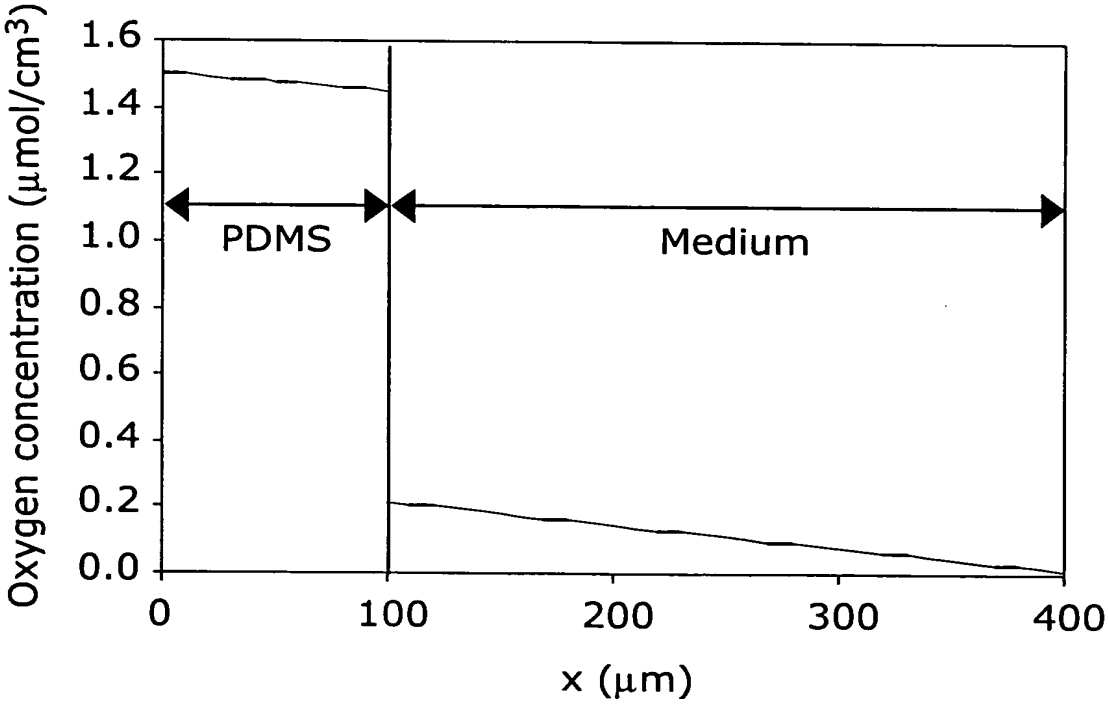
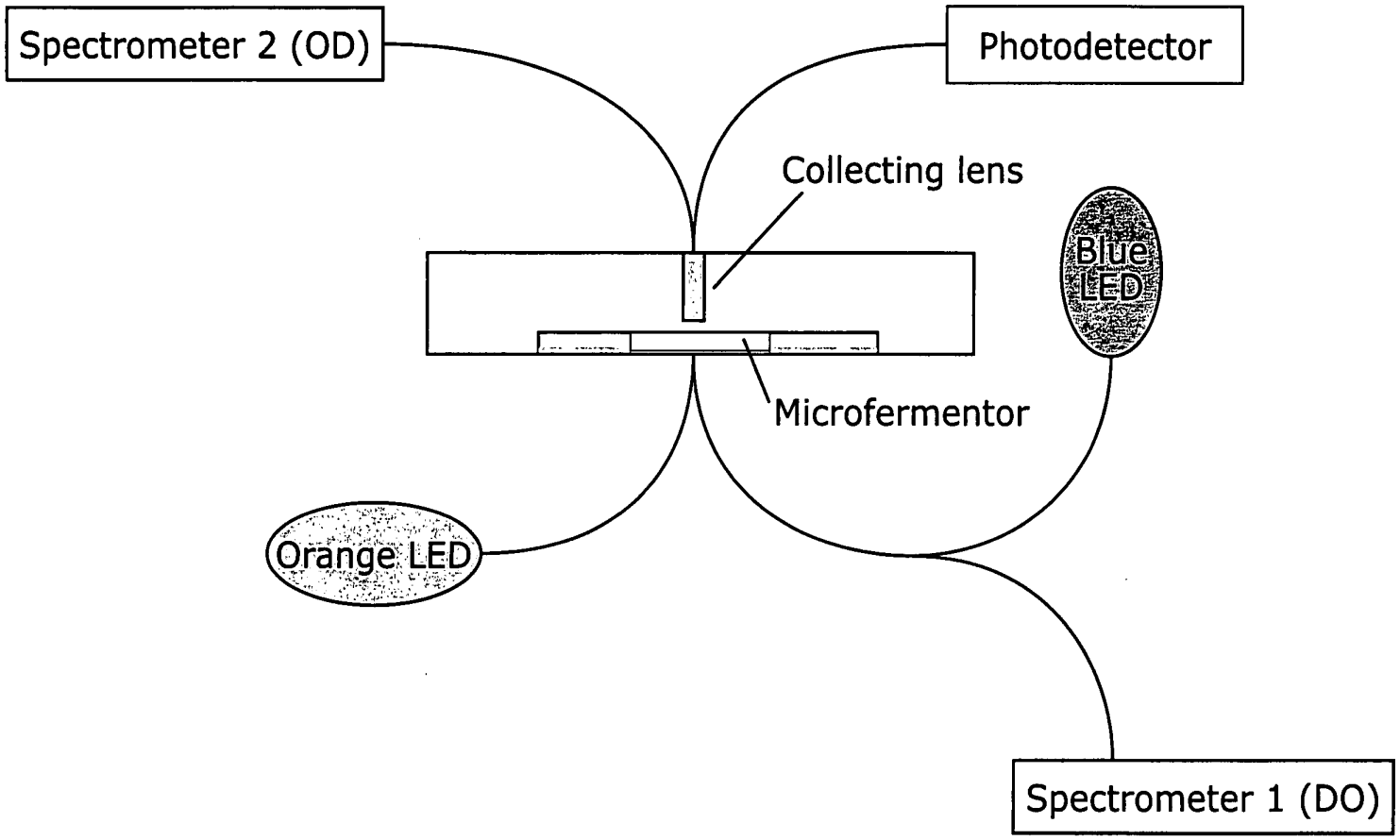


FIG. 13B

Microfermentor setup



Optical density:	Transmittance measurement at ~600nm
Dissolved oxygen:	Fluorescence quenching of fluorophone

FIG. 14

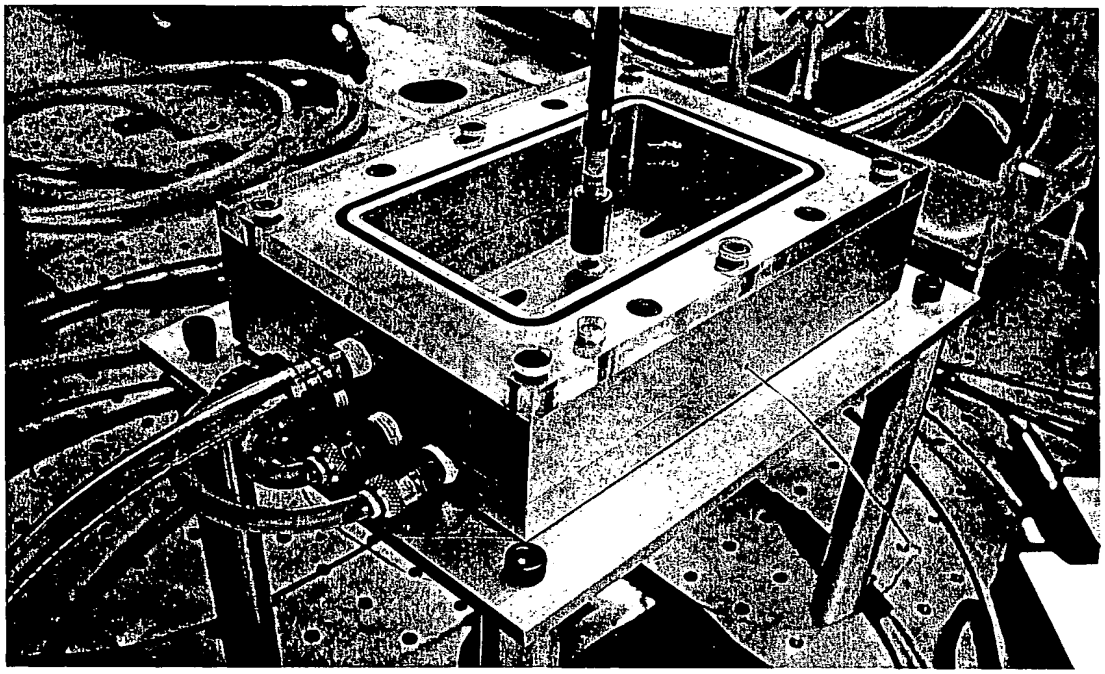


FIG. 15A

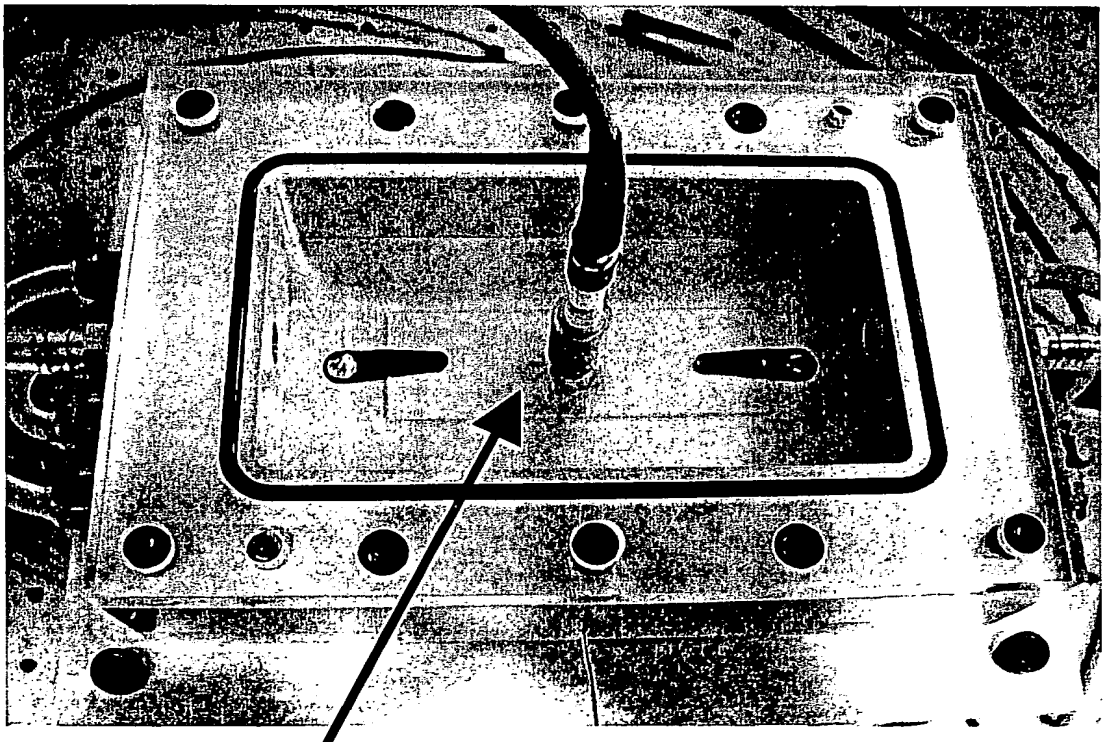
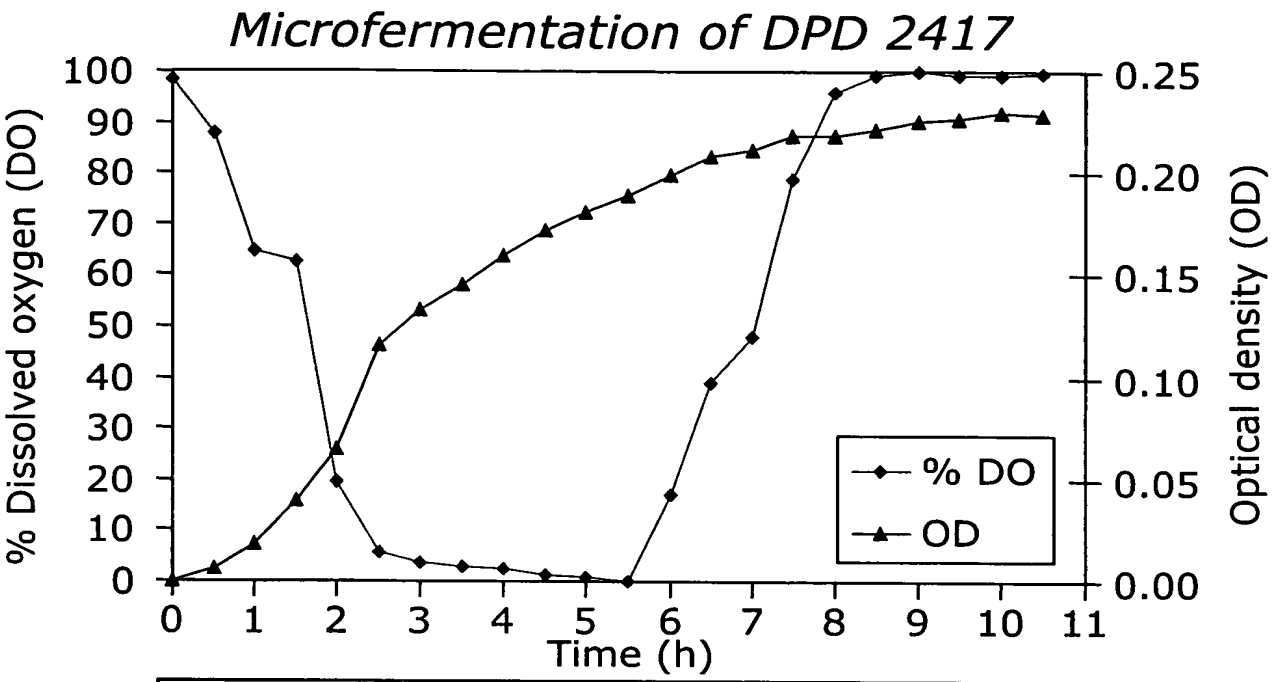
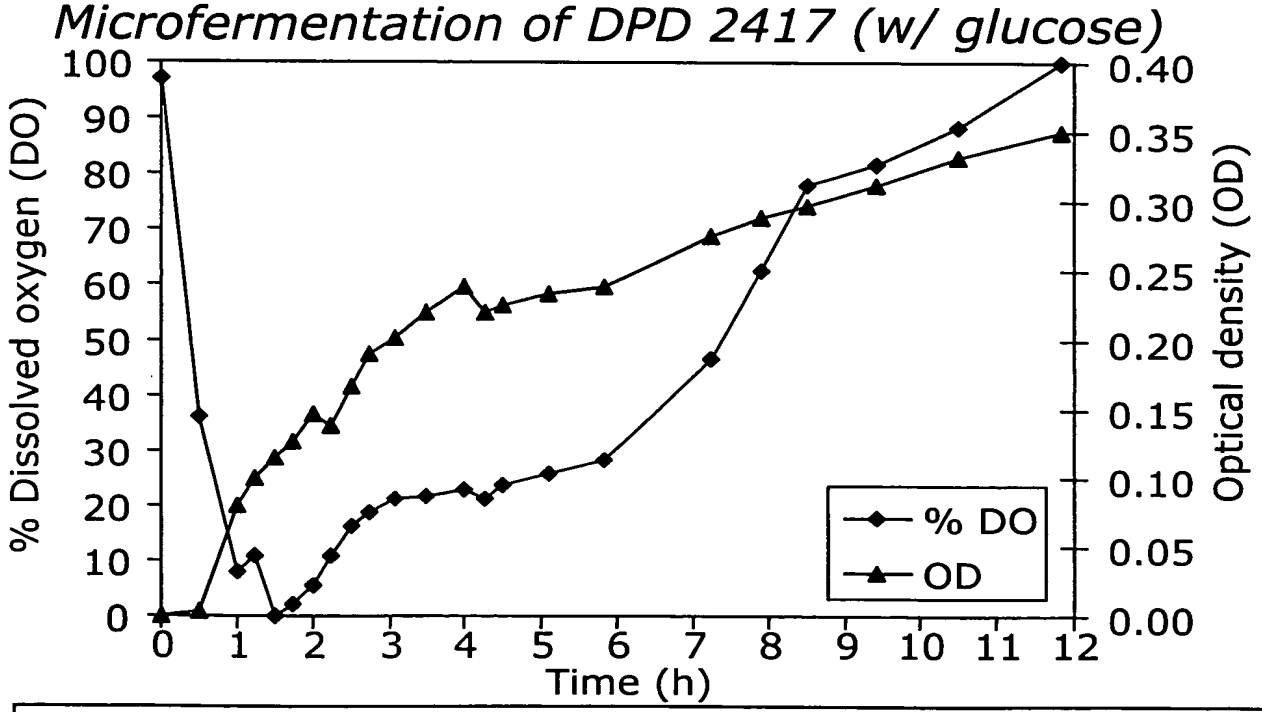


FIG. 15B



Conditions:	37°C, 5% inoculum, LB+amp
Light source for OD:	orange LED (609nm peak)
Sterilization:	60s under UV (254nm)

FIG. 16



Conditions:	37°C, 5% inoculum, LB+amp +30g/L glucose
Light source for OD:	orange LED (609nm peak)
Sterilization:	60s under UV (254nm)

FIG. 17

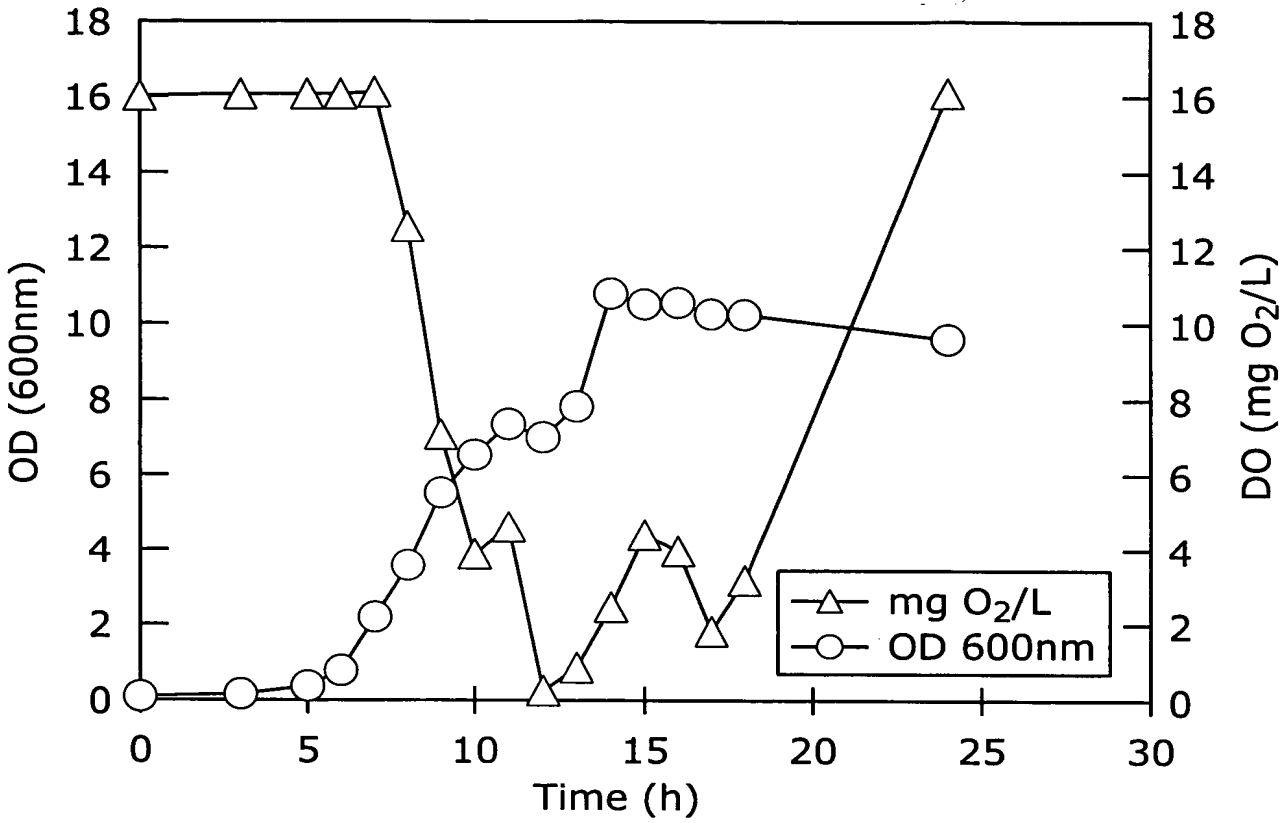


FIG. 18A

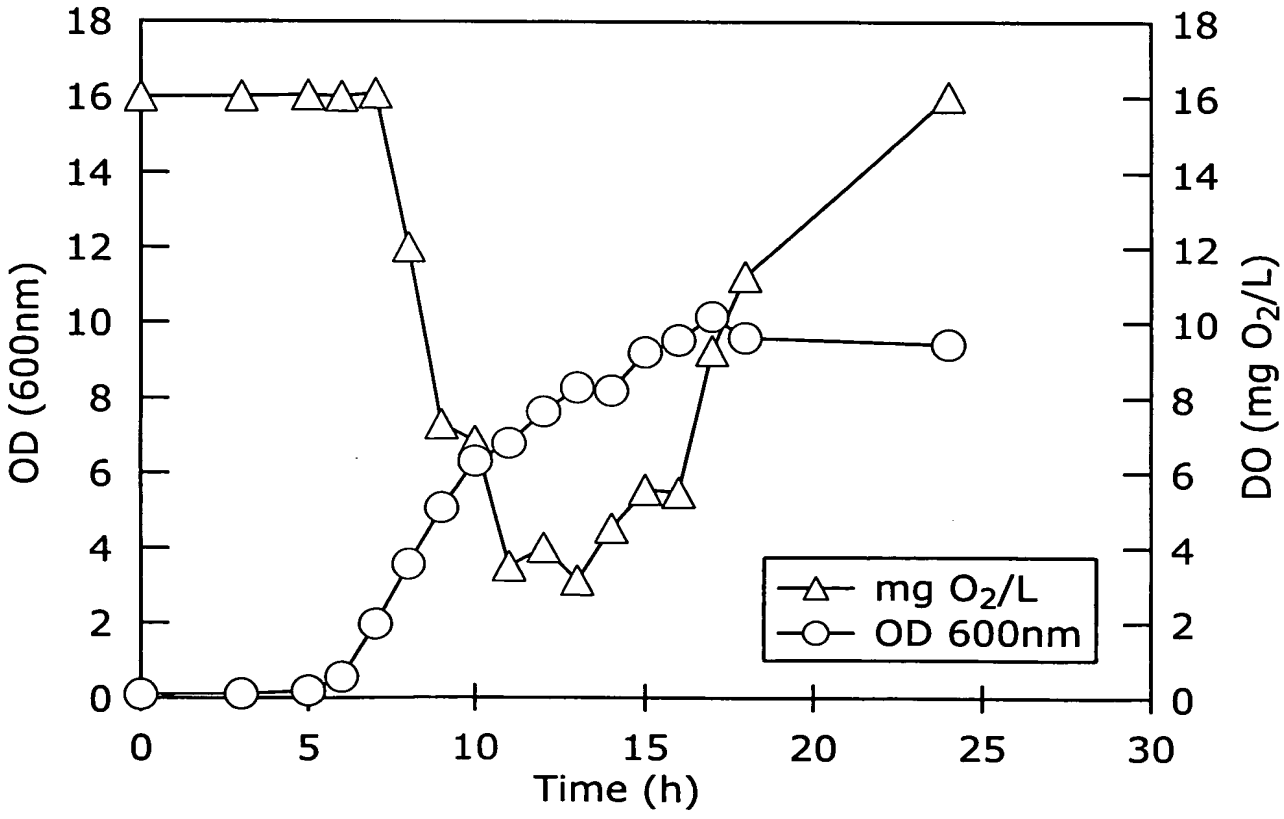


FIG. 18B

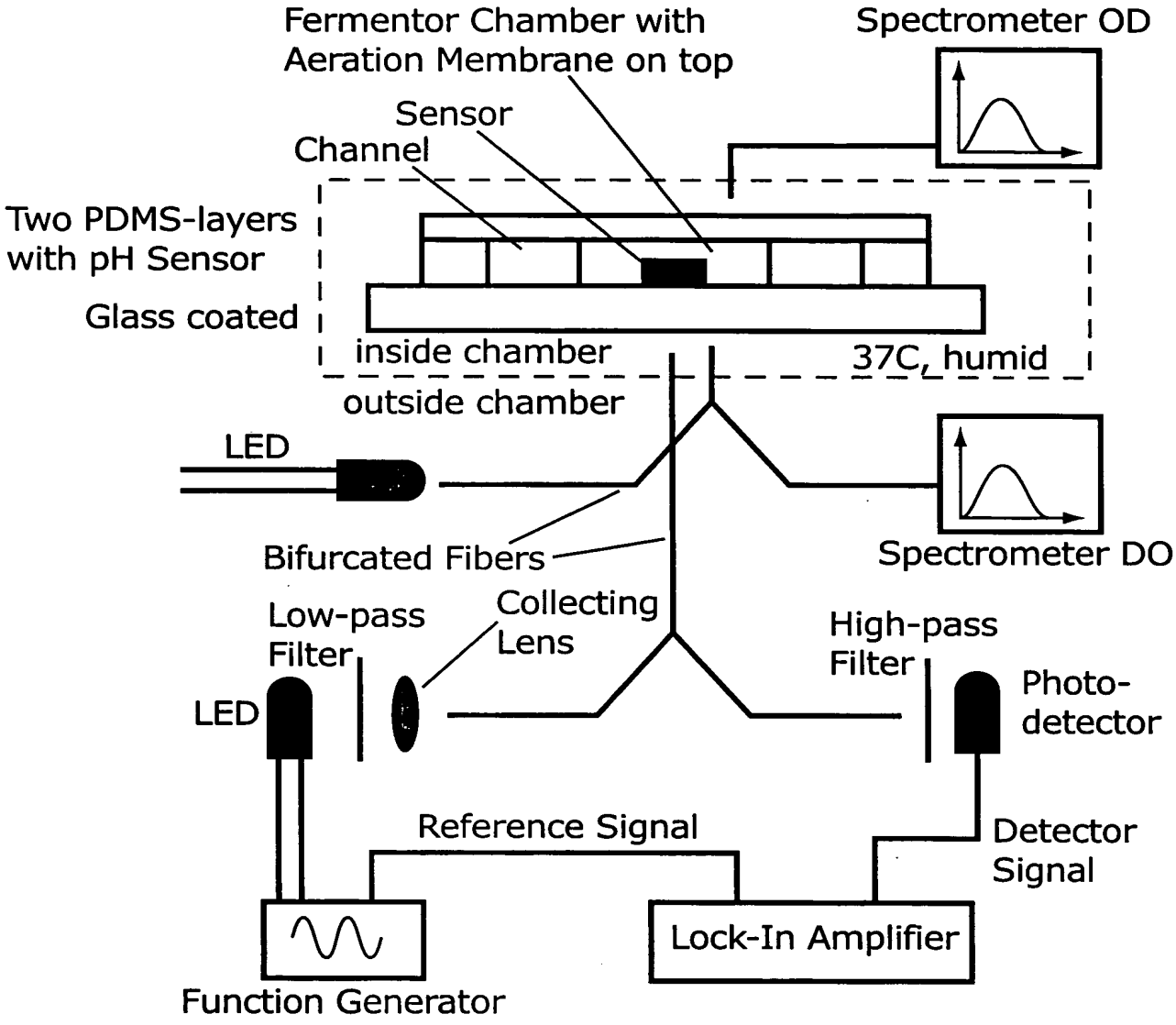

Fermentor Chamber with
Aeration Membrane on top
Spectrometer OD
Sensor
Channel
Two PDMS-layers
with pH Sensor
Glass coated
inside chamber
37C, humid
outside chamber
LED
Bifurcated Fibers
Spectrometer DO
Low-pass
Filter
Collecting
Lens
High-pass
Filter
LED
Photo-
detector
Reference Signal
Detector
Signal
Lock-In Amplifier
Function Generator

FIG. 19

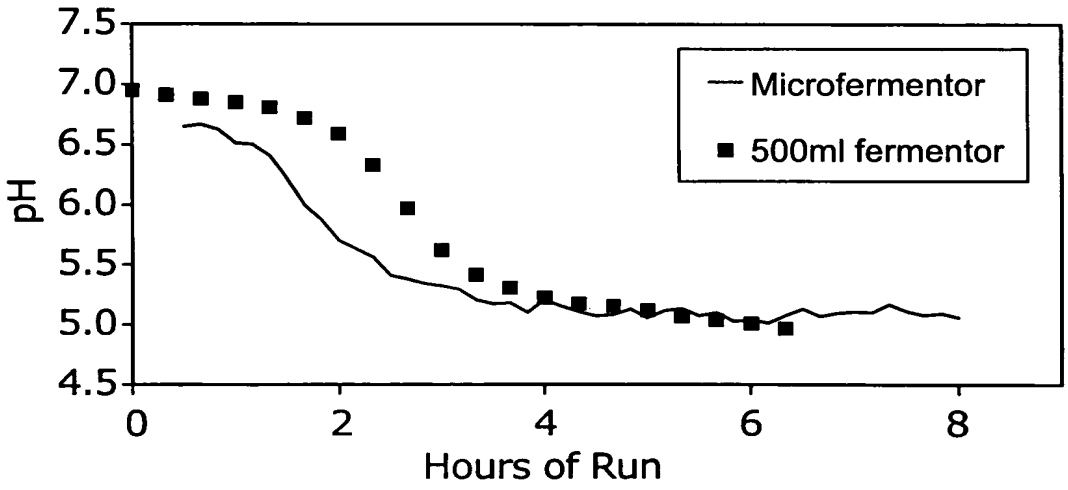

7.5
7.0
6.5
6.0
5.5
5.0
4.5
pH
0
2
4
6
8
Hours of Run
Microfermentor
500ml fermentor

FIG. 20

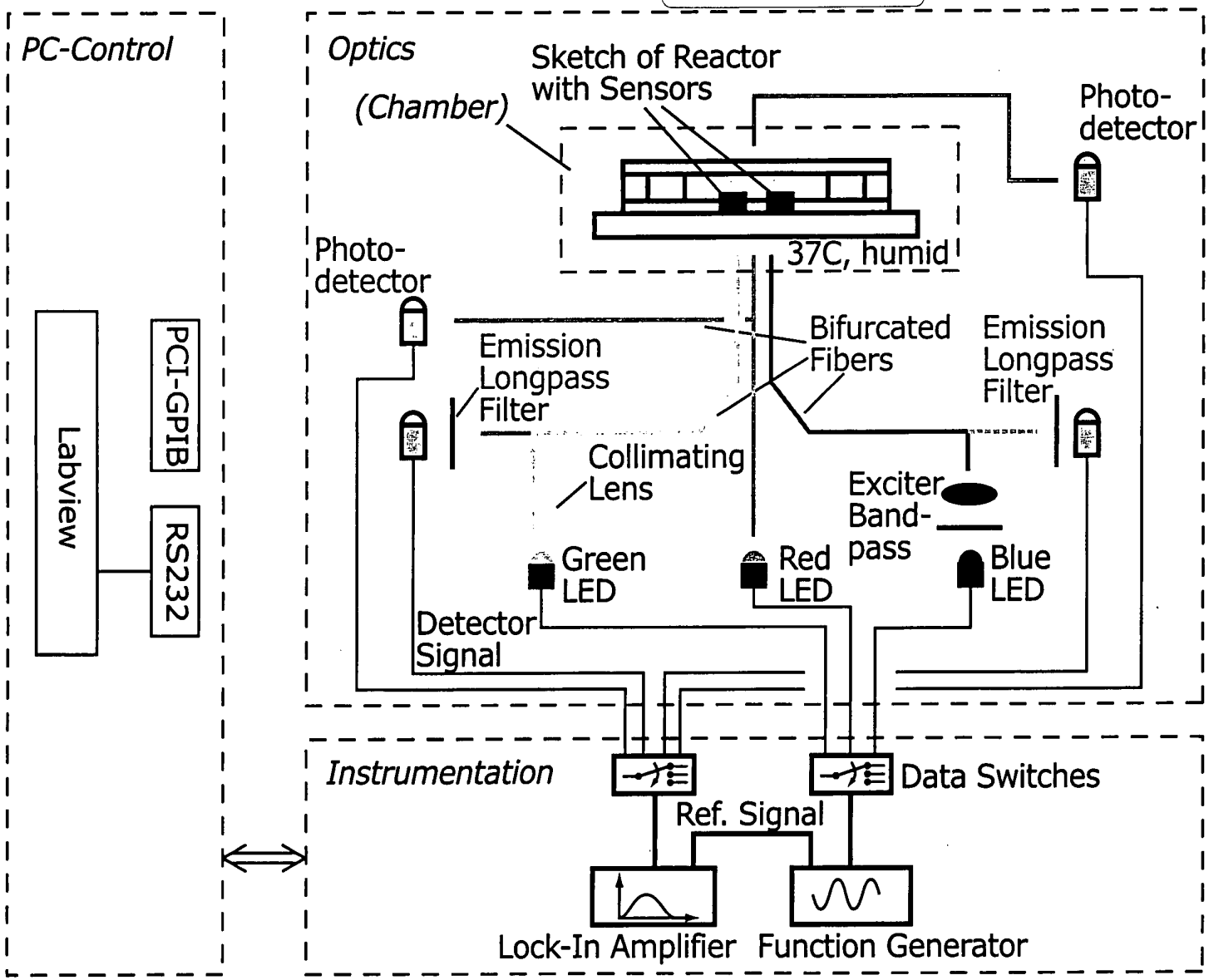

PC-Control
Labview
PCI-GPIB
RS232
Optics
Sketch of Reactor with Sensors
(Chamber)
Photo-detector
37C, humid
Photo-detector
Bifurcated Fibers
Emission Longpass Filter
Emission Longpass Filter
Collimating Lens
Exciter Band-pass
Green LED
Red LED
Blue LED
Detector Signal
Instrumentation
Data Switches
Ref. Signal
Lock-In Amplifier
Function Generator

FIG. 21

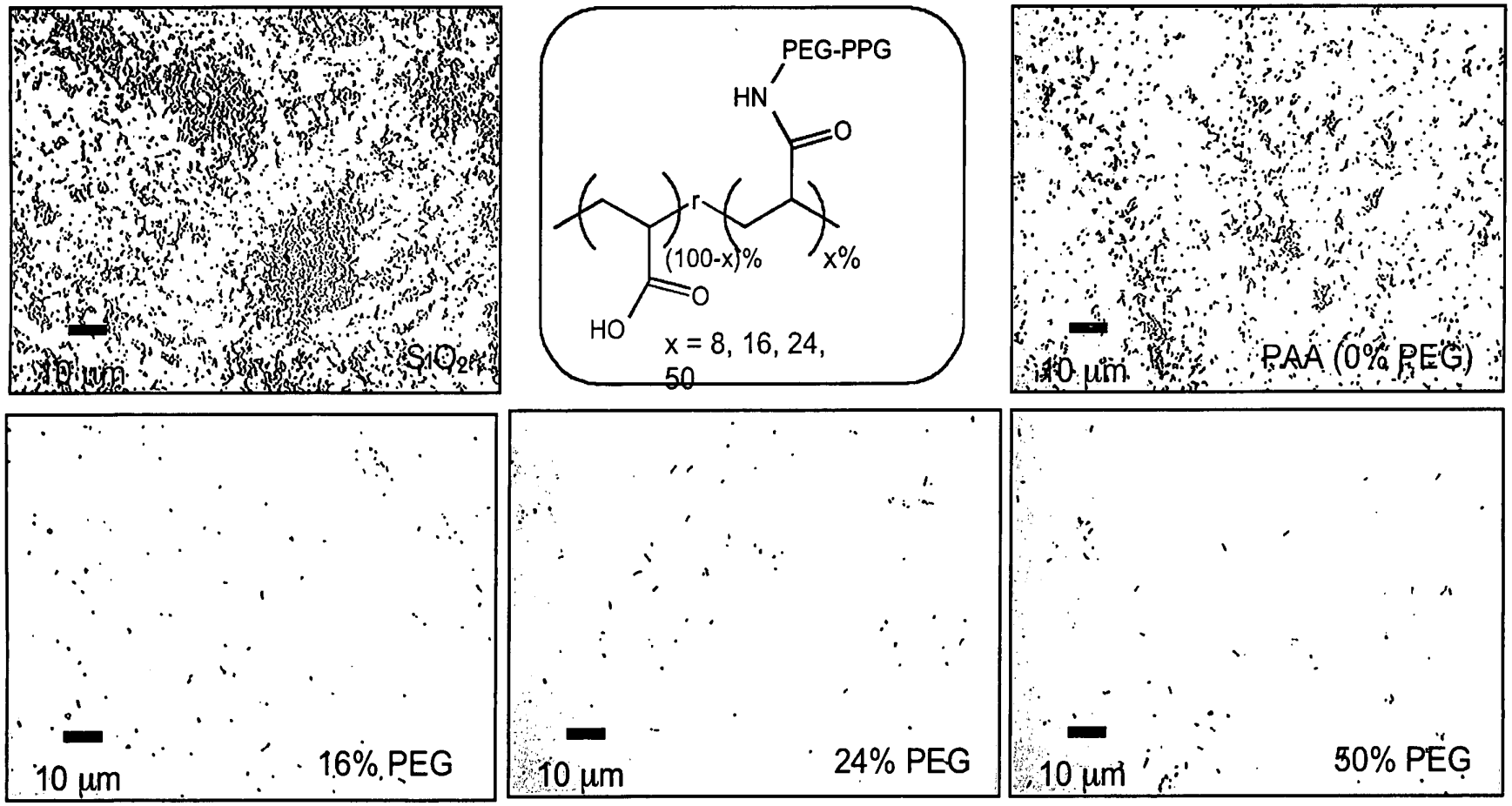

10 µm
SiO2
PEG-PPG
HN
O
r
(100-x)%
x%
O
HO
x = 8, 16, 24, 50
10 µm
PAA (0% PEG)
10 µm
16% PEG
10 µm
24% PEG
10 µm
50% PEG

FIG. 22

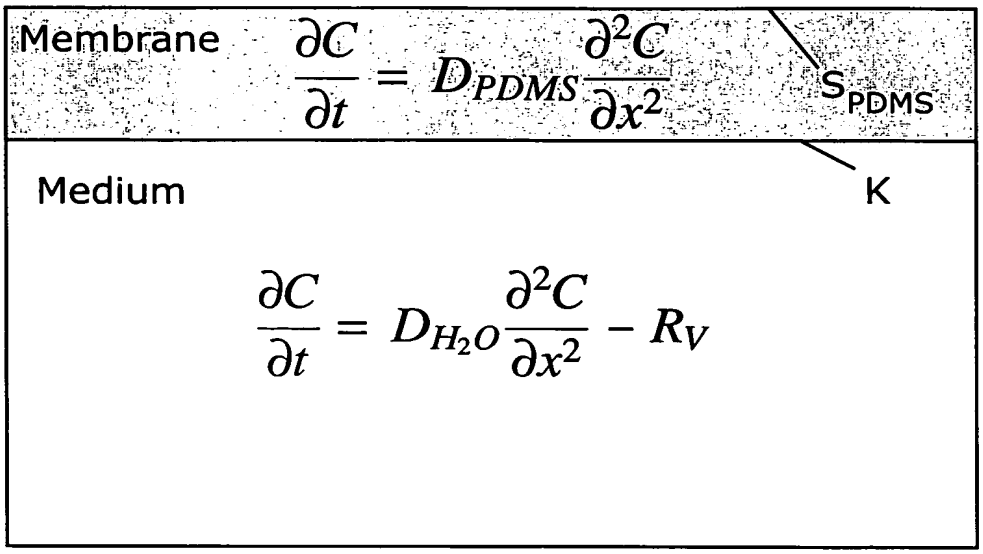

Membrane
$\frac{\partial C}{\partial t} = D_{PDMS}\frac{\partial^2 C}{\partial x^2}$
S_{PDMS}
Medium
K
$\frac{\partial C}{\partial t} = D_{H_2O}\frac{\partial^2 C}{\partial x^2} - R_V$

FIG. 23

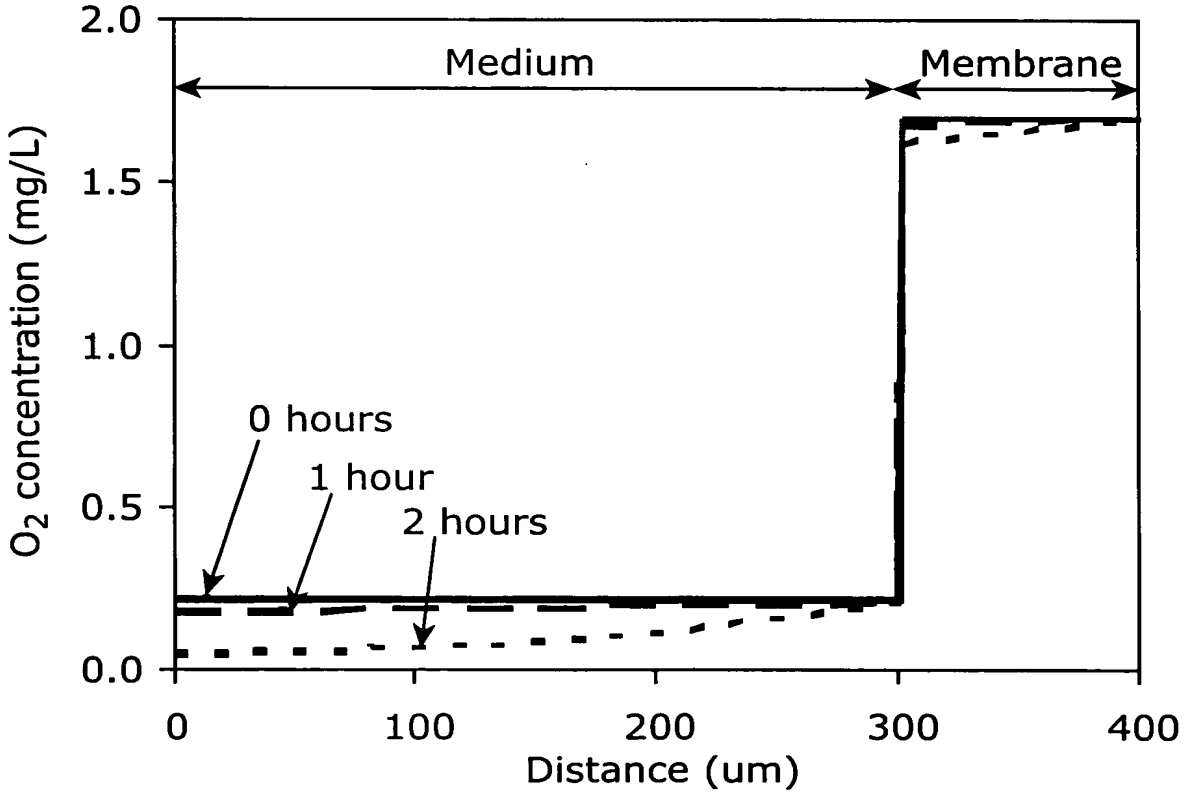

2.0
1.5
1.0
0.5
0.0
O_2 concentration (mg/L)
Medium
Membrane
0 hours
1 hour
2 hours
0
100
200
300
400
Distance (um)

FIG. 24

Figure 25A

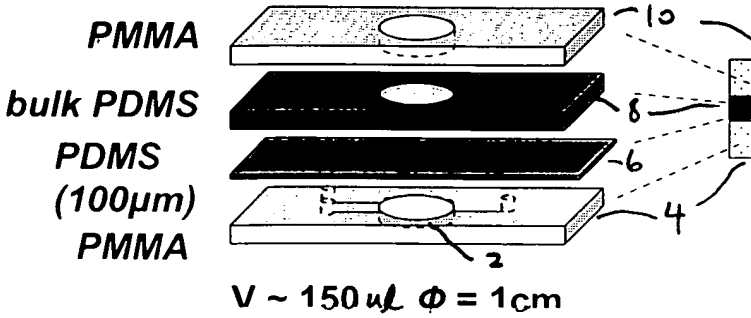


Figure 25B

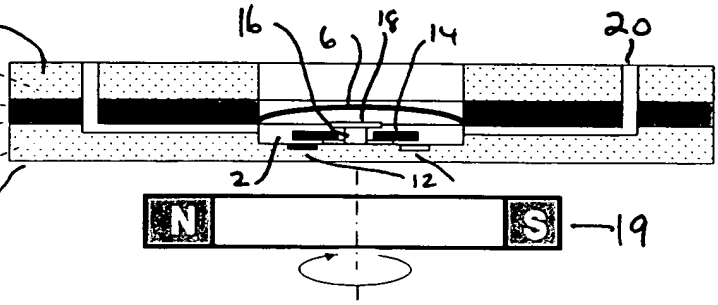
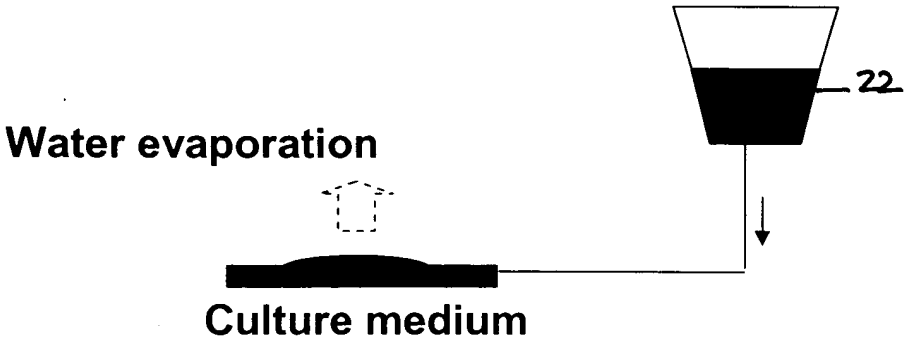


Figure 25C



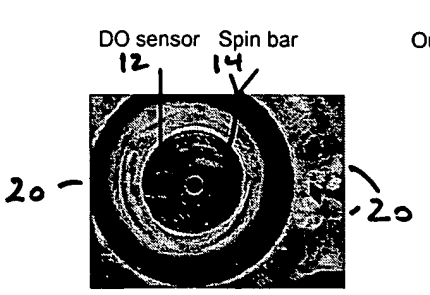


Figure 26A

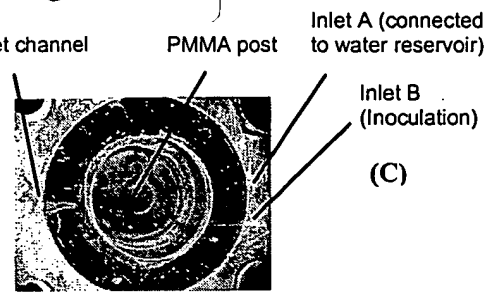
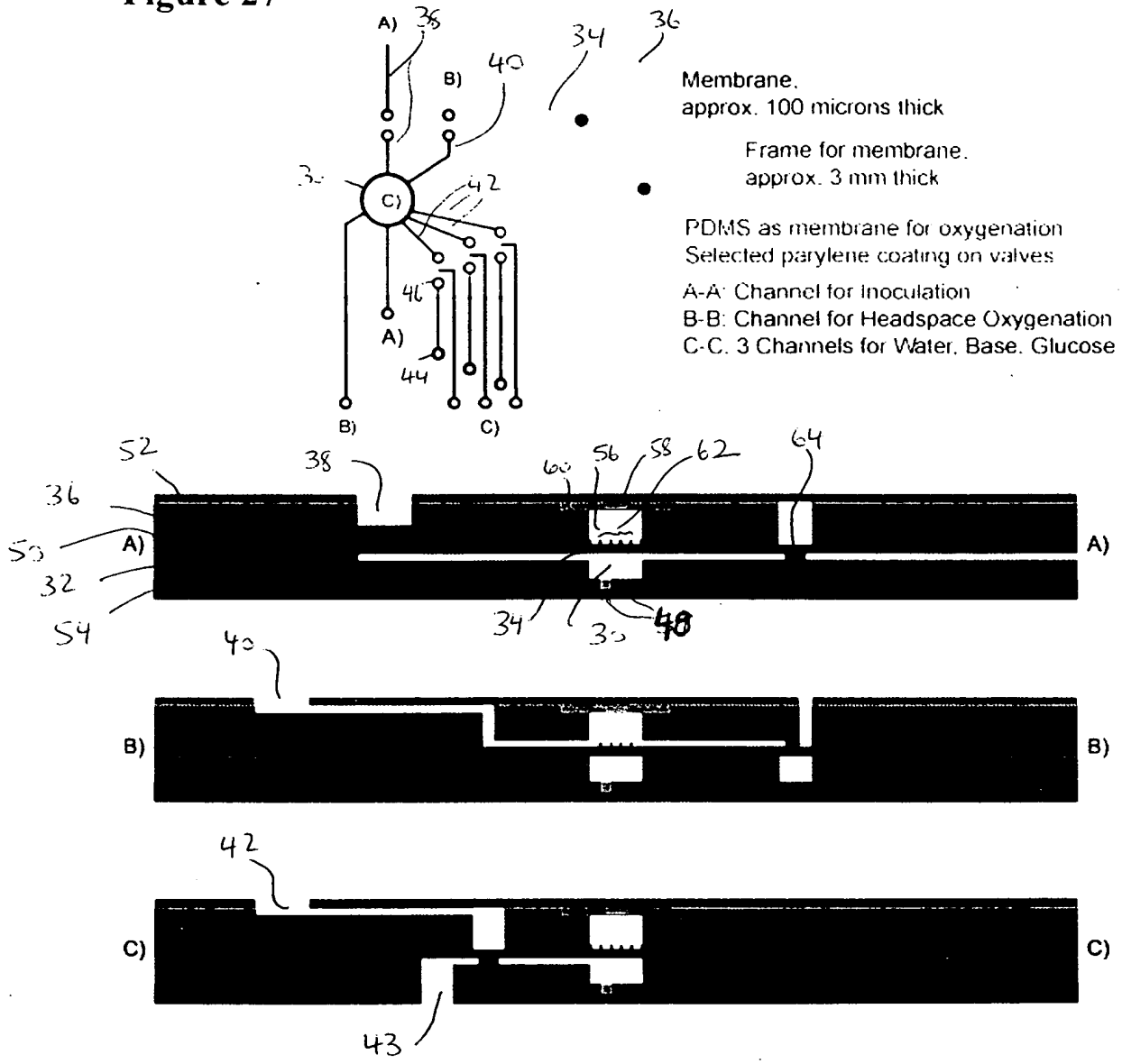


Figure 26B

Figure 27



Figures 28A – 28C

Figure 29

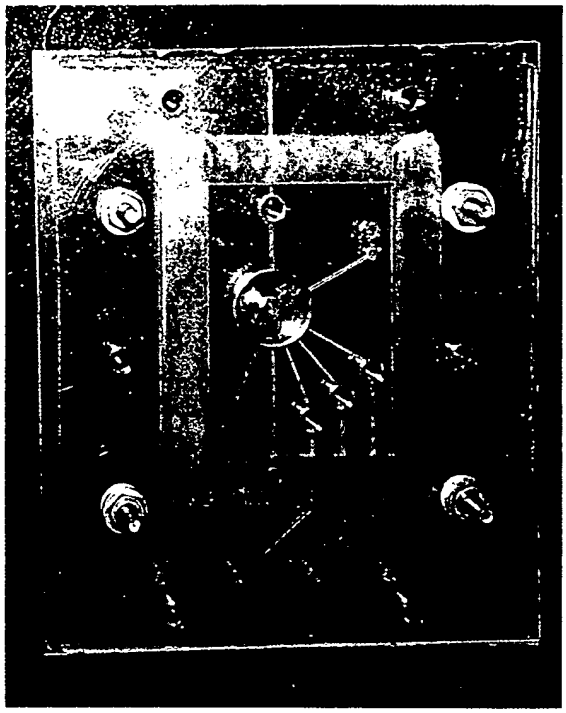
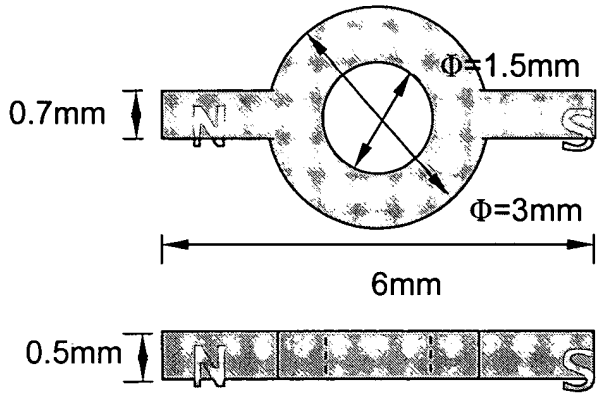


Figure 30

A



B

Figure 31

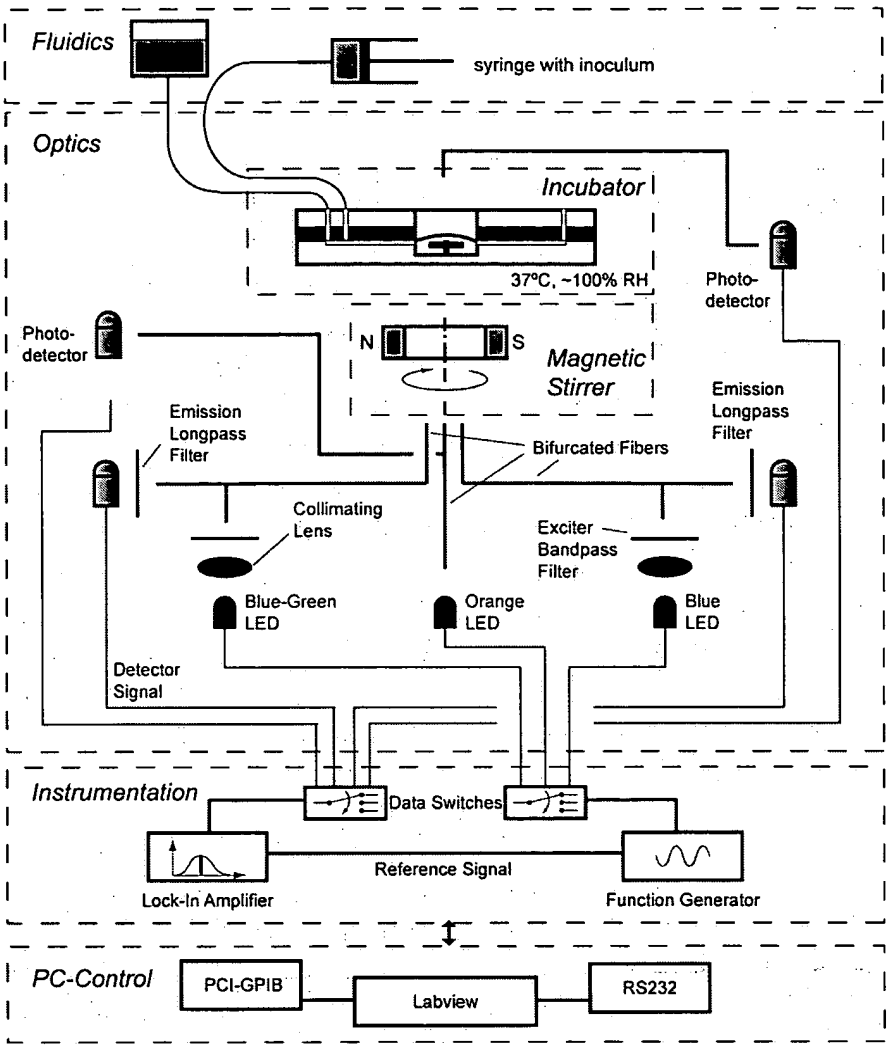

Fluidics
syringe with inoculum
Optics
Incubator
37°C, ~100% RH
Photo-detector
Photo-detector
N
S
Magnetic Stirrer
Emission Longpass Filter
Bifurcated Fibers
Emission Longpass Filter
Collimating Lens
Exciter Bandpass Filter
Blue-Green LED
Orange LED
Blue LED
Detector Signal
Instrumentation
Data Switches
Reference Signal
Lock-In Amplifier
Function Generator
PC-Control
PCI-GPIB
Labview
RS232

Figure 32A

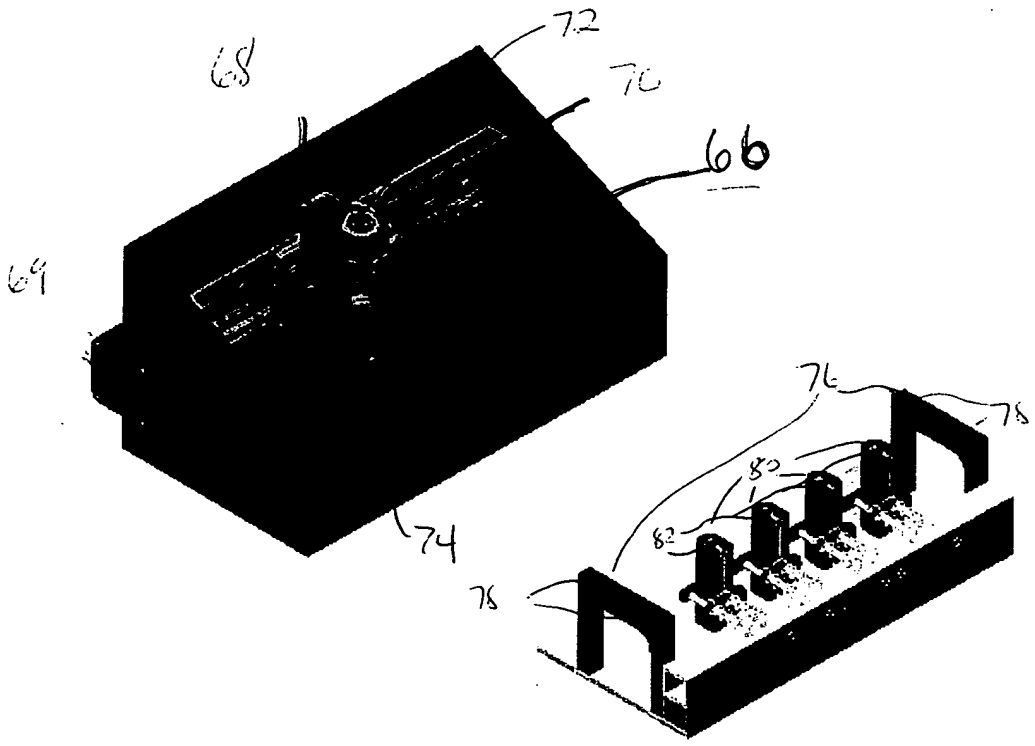


Figure 32D

FIGURE 32B

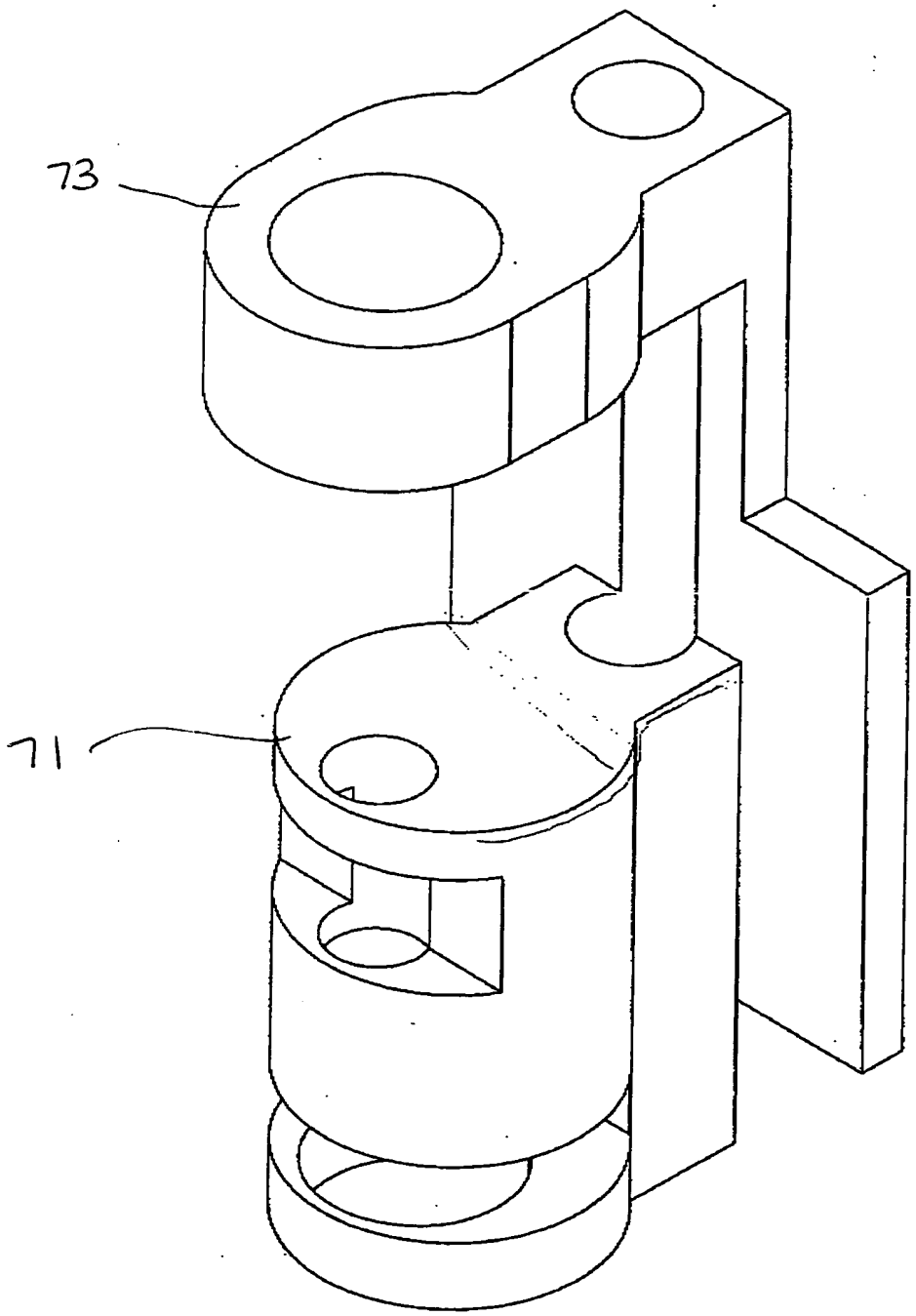
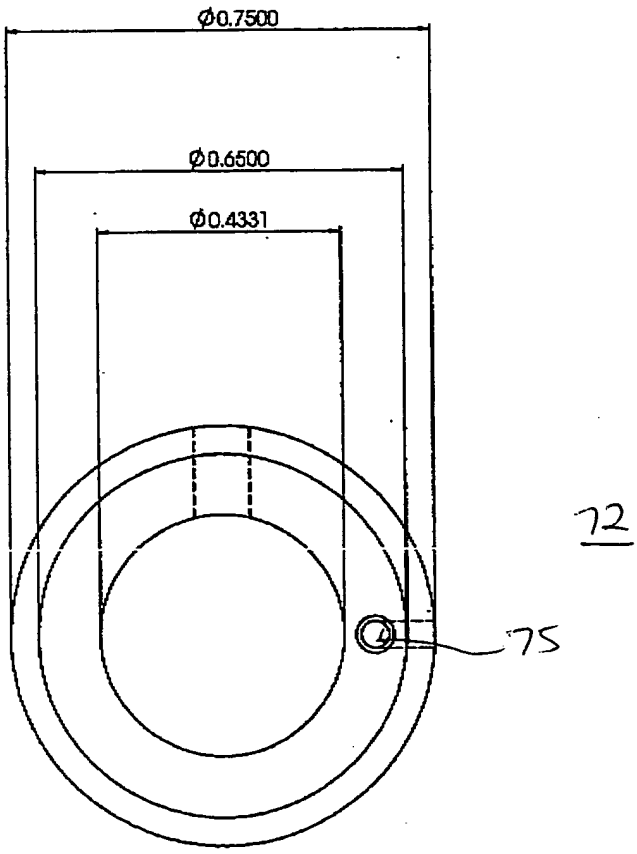


FIGURE 32C



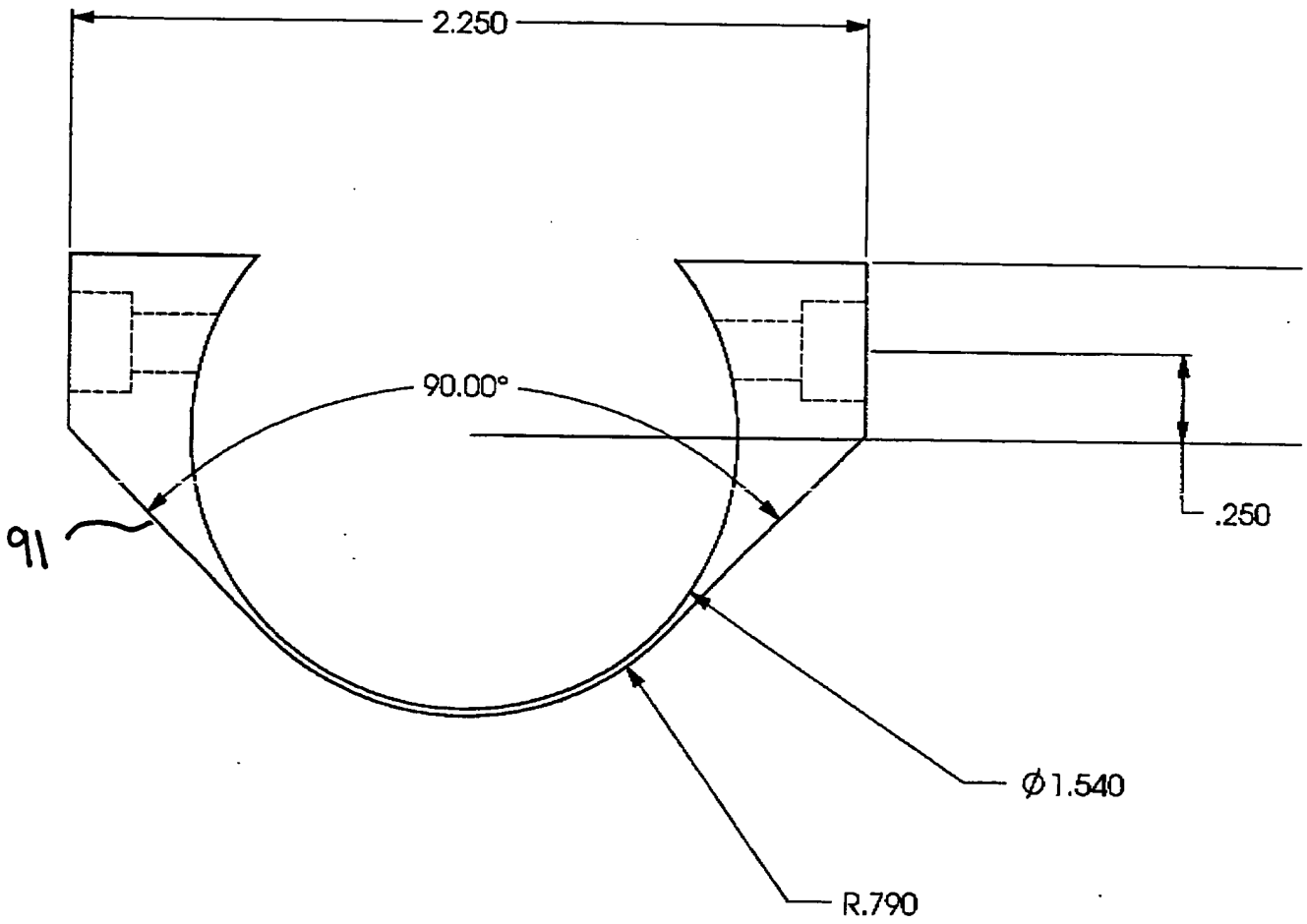


FIGURE 32E

Figure 33

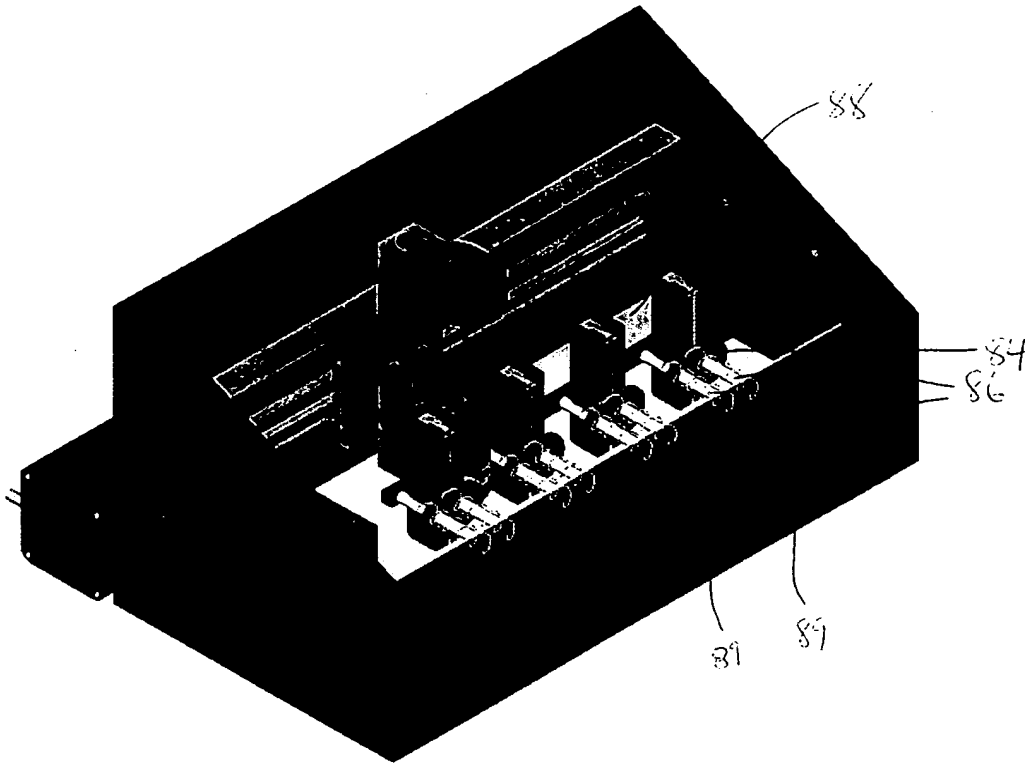


Figure 34A

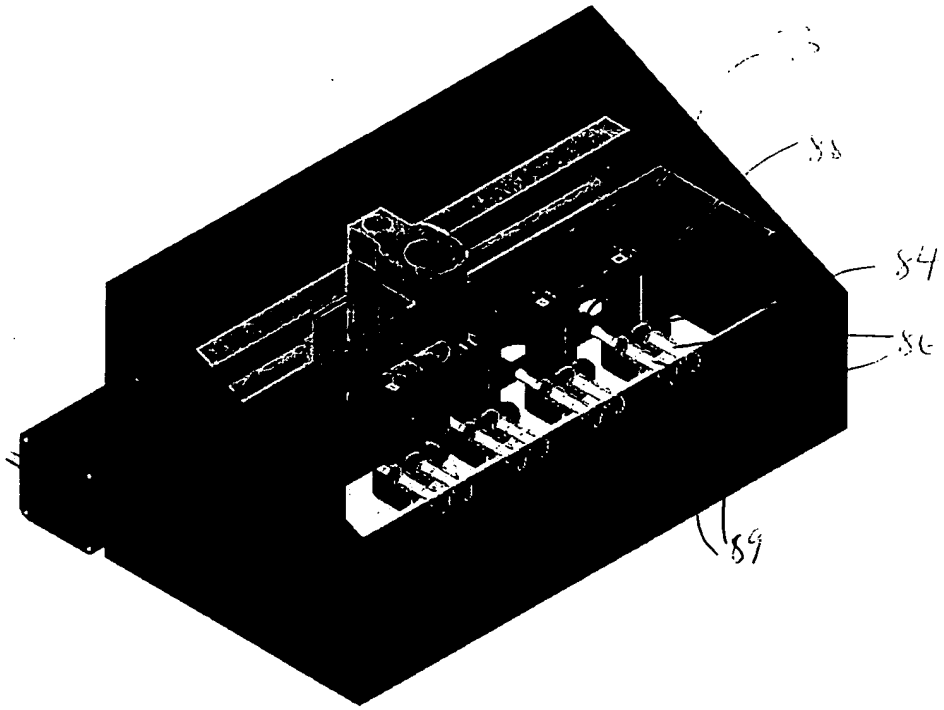


Figure 34B

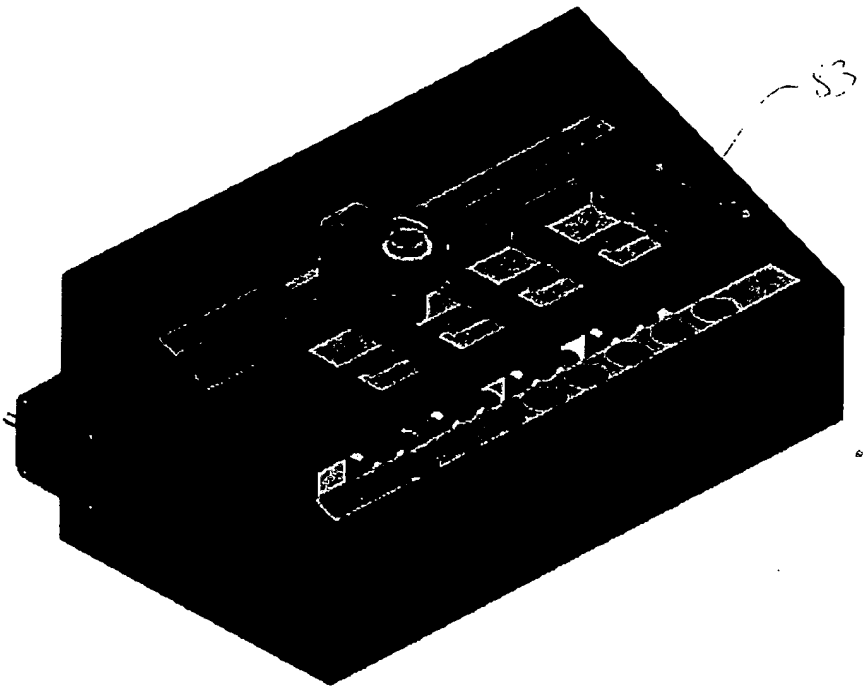


Figure 35A

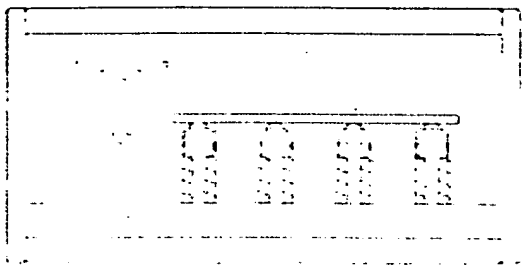


Figure 35B

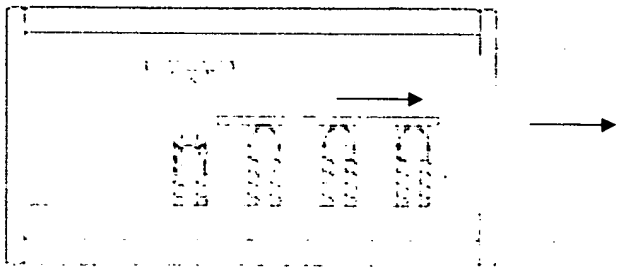


Figure 36

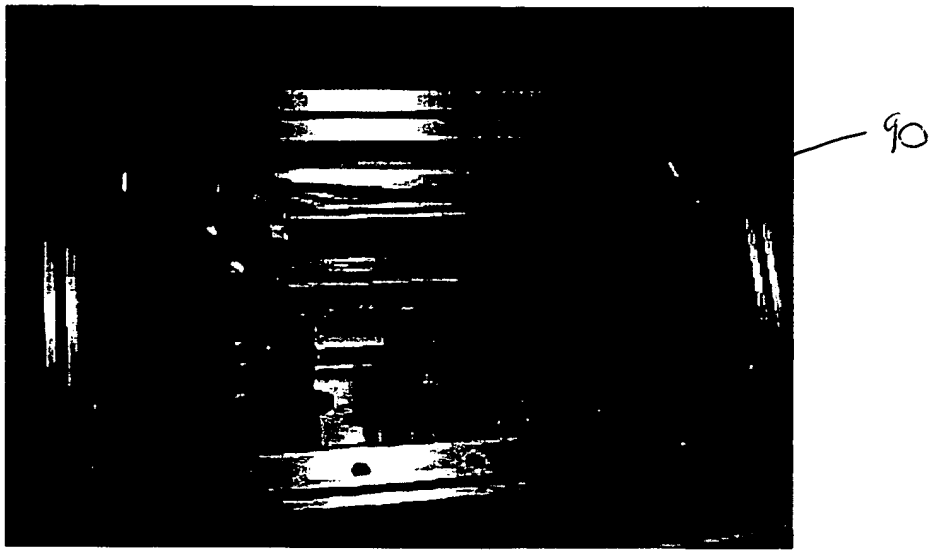


Figure 37

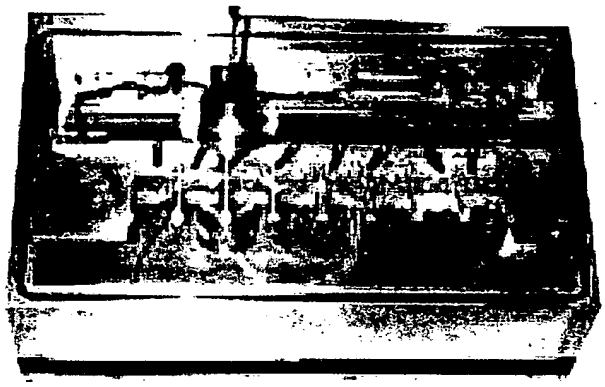
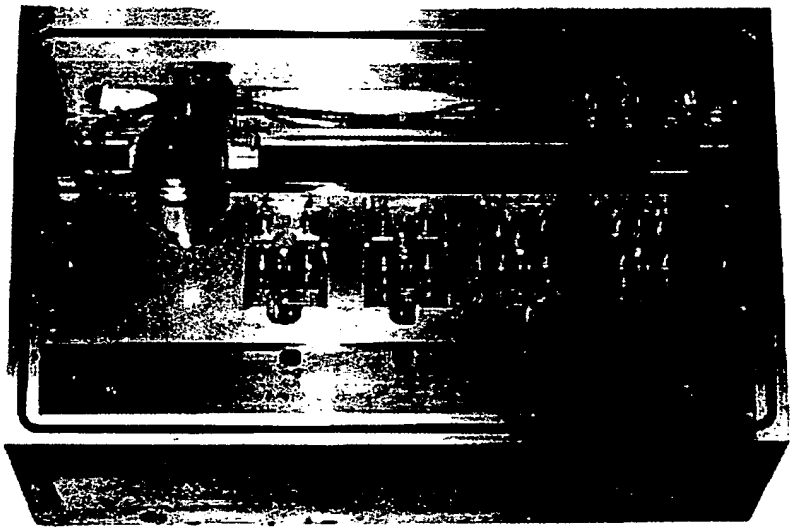


Figure 39



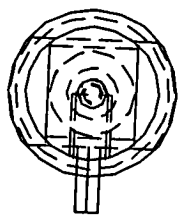


FIGURE 38D

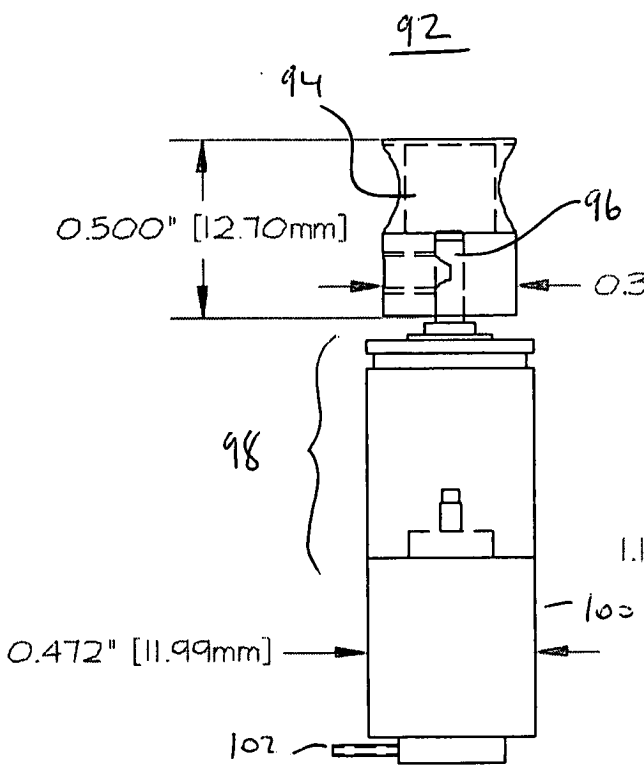


FIGURE 38A

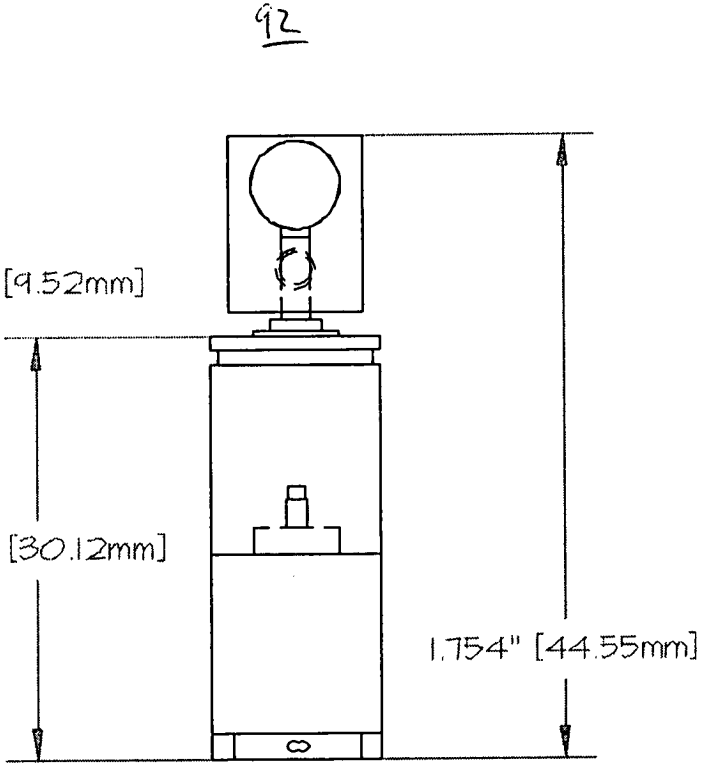


FIGURE 38B

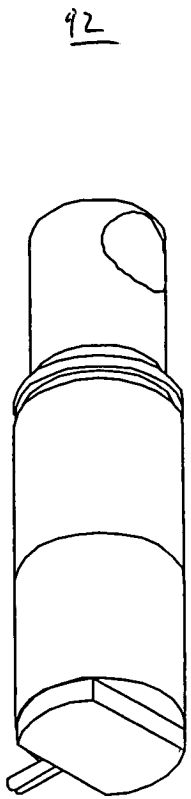


FIGURE 38C

FIGURE 40A

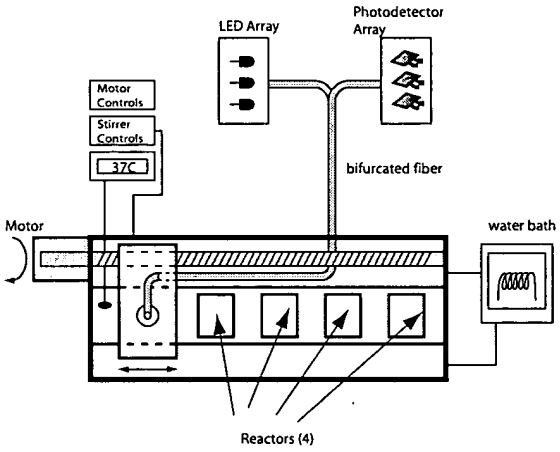

LED Array
Photodetector Array
Motor Controls
Stirrer Controls
37C
bifurcated fiber
Motor
water bath
Reactors (4)

Figure 40B

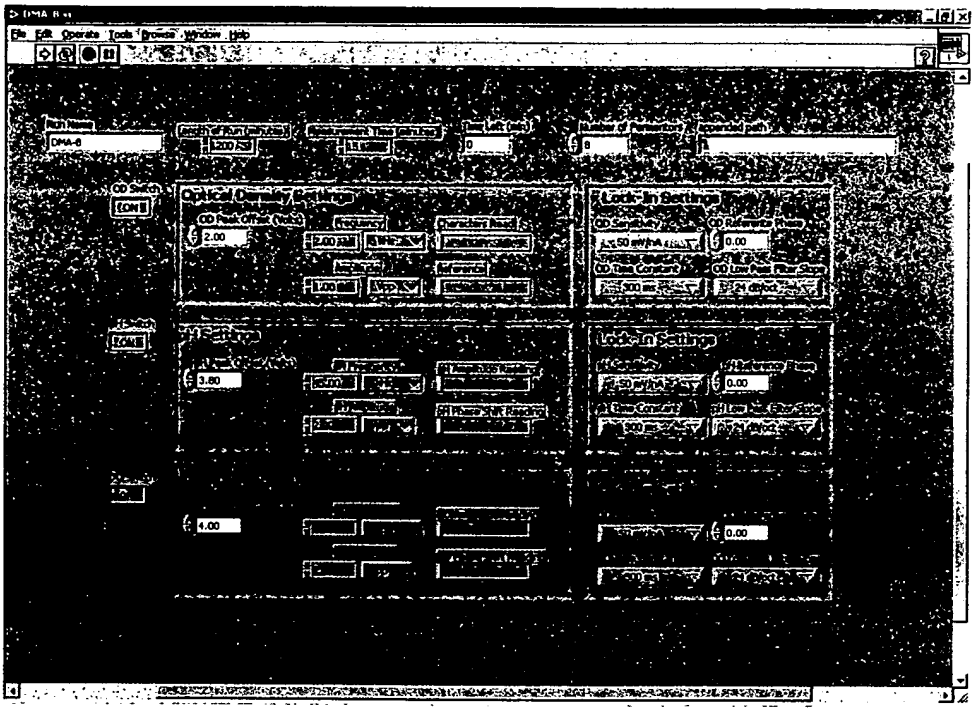

File Edit Operate Tools Browse Window Help
DMA-8
Lock-In Settings
Lock-In Settings

Figure 41

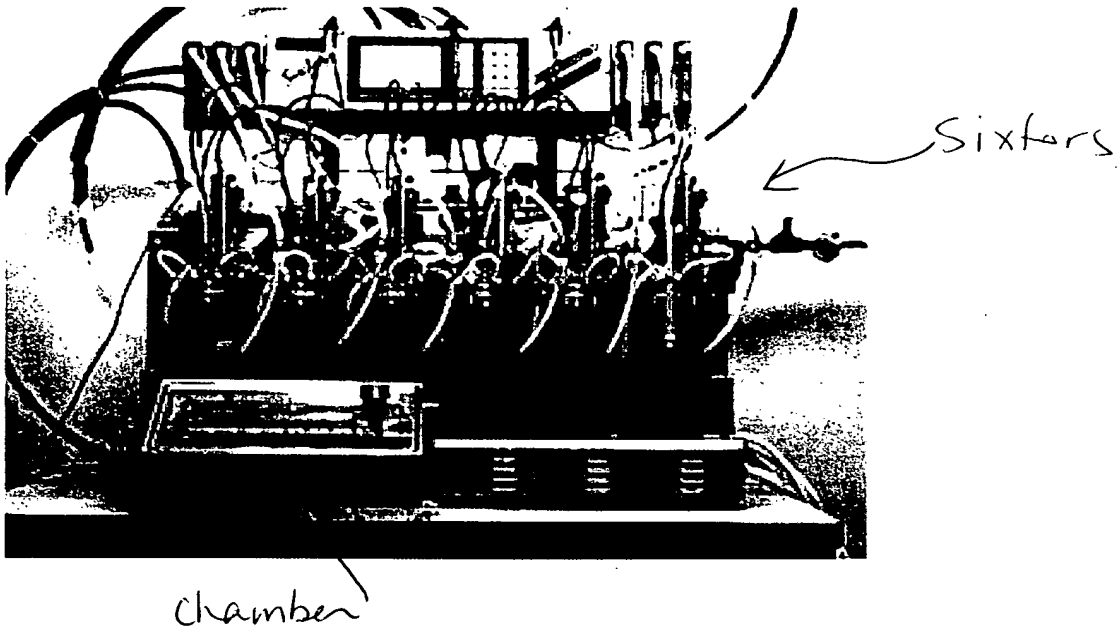
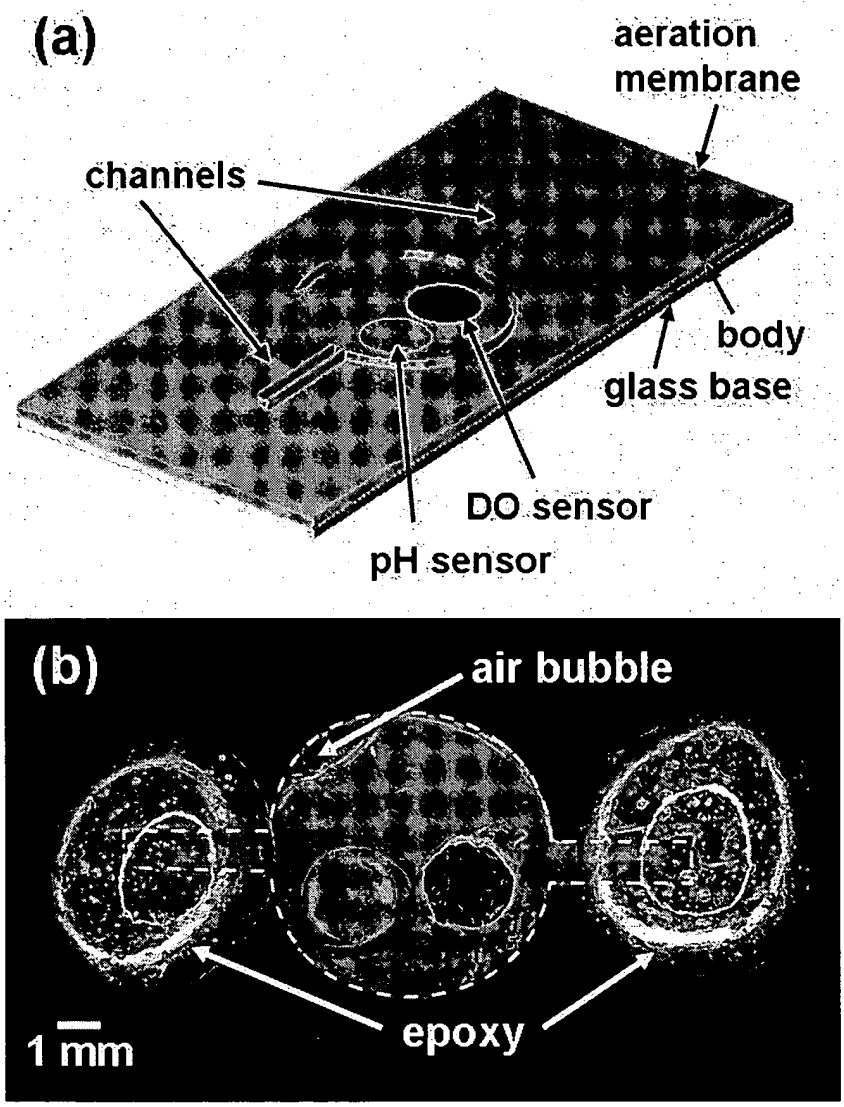


Figure 42


(a)
aeration membrane
channels
body
glass base
DO sensor
pH sensor
(b)
air bubble
epoxy
1 mm

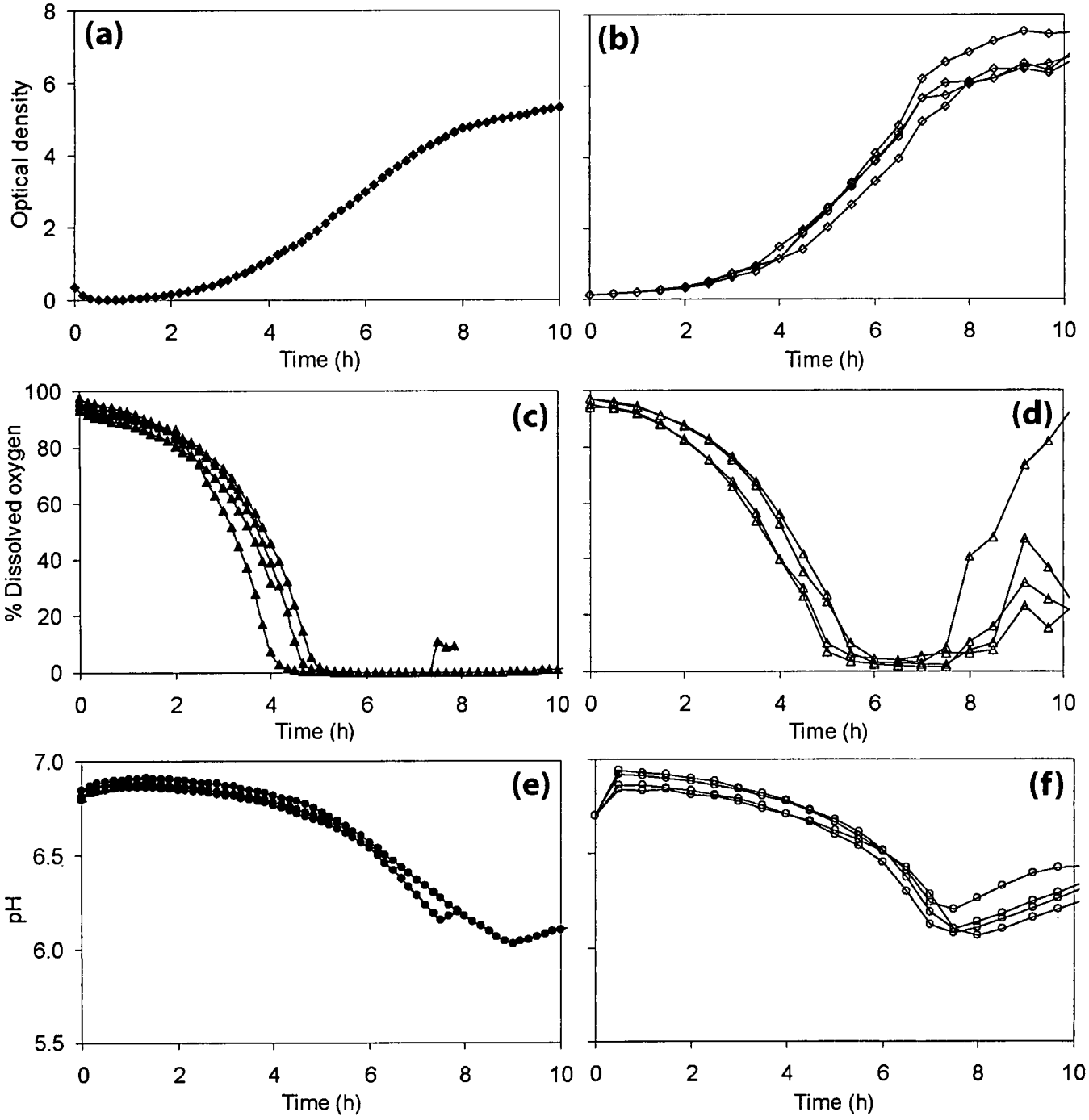

(a)
(b)
(c)
(d)
(e)
(f)
Optical density
% Dissolved oxygen
pH
Time (h)
0
2
4
6
8
10
5.5
6.0
6.5
7.0
20
40
60
80
100

FIGURE 43

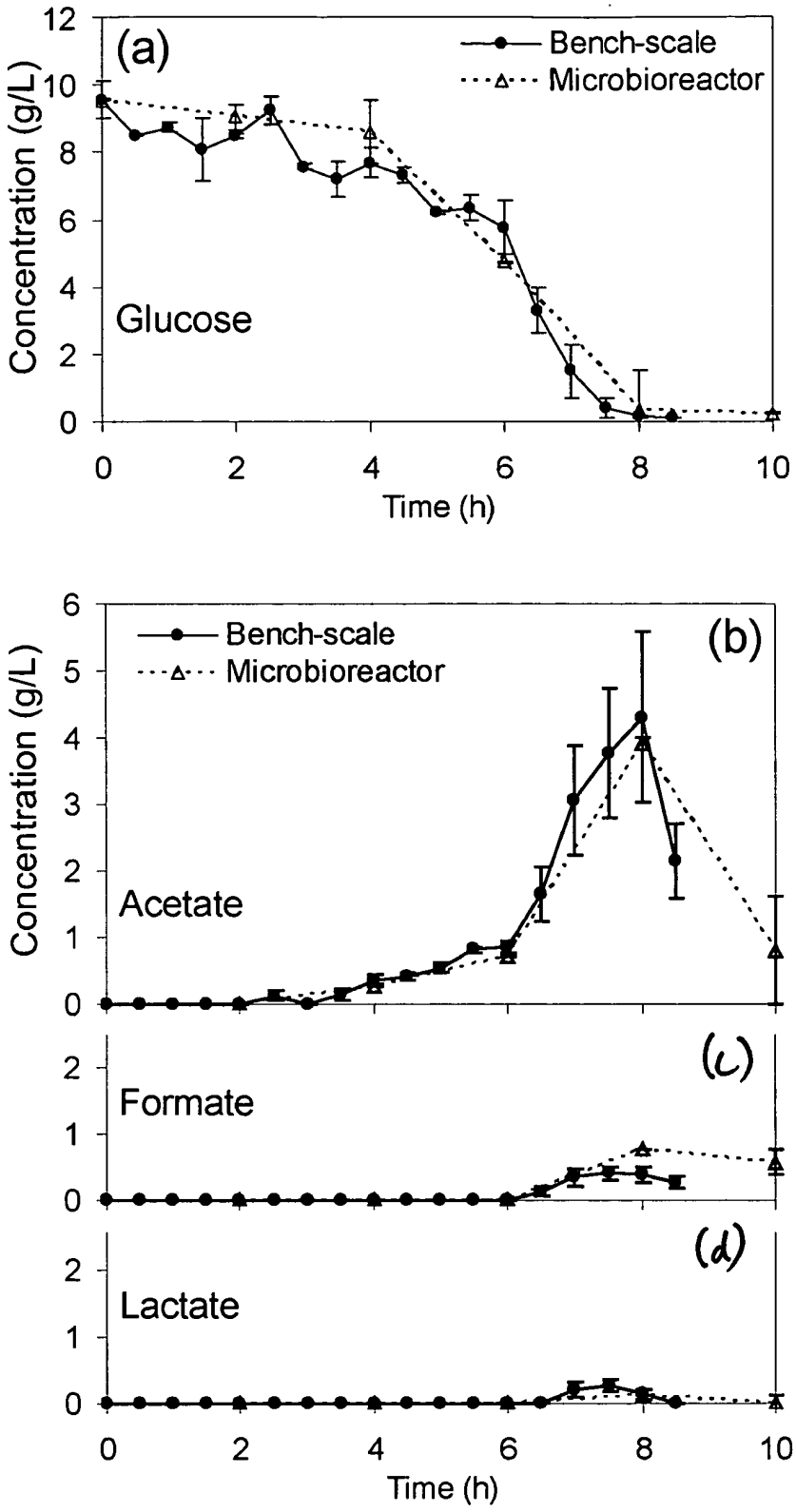

(a)
Bench-scale
Microbioreactor
Concentration (g/L)
Glucose
Time (h)
(b)
Acetate
(c)
Formate
(d)
Lactate

FIGURE 44

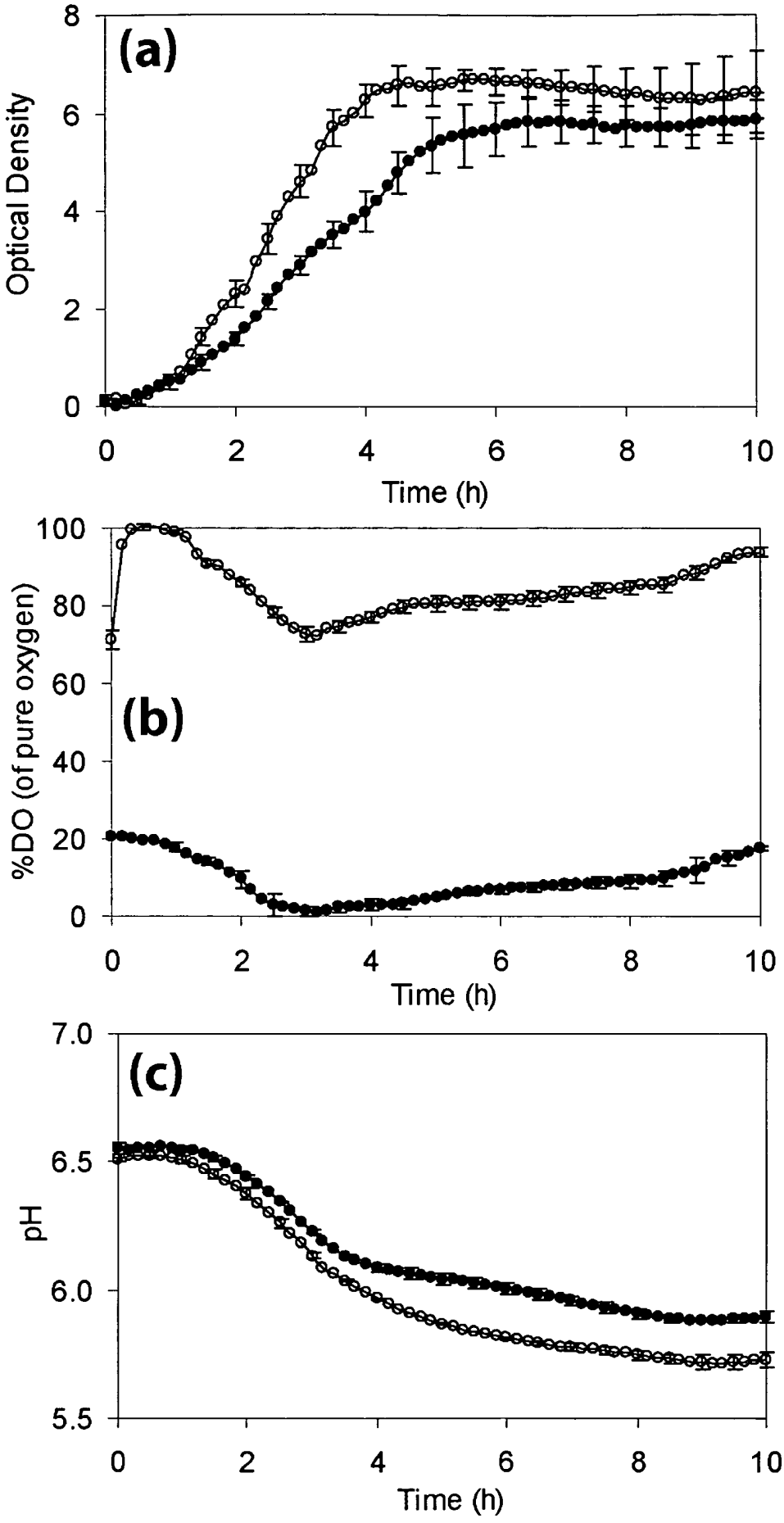

(a)
Optical Density
0
2
4
6
8
0
2
4
6
8
10
Time (h)
(b)
%DO (of pure oxygen)
0
20
40
60
80
100
0
2
4
6
8
10
Time (h)
(c)
pH
5.5
6.0
6.5
7.0
0
2
4
6
8
10
Time (h)
Oxygen
Air

FIGURE 45

Figure 46A

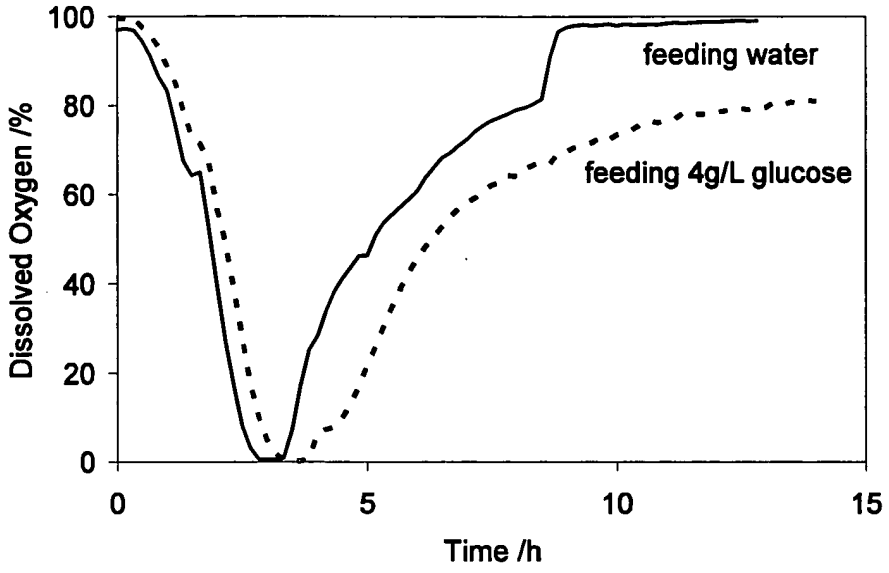


Figure 46B

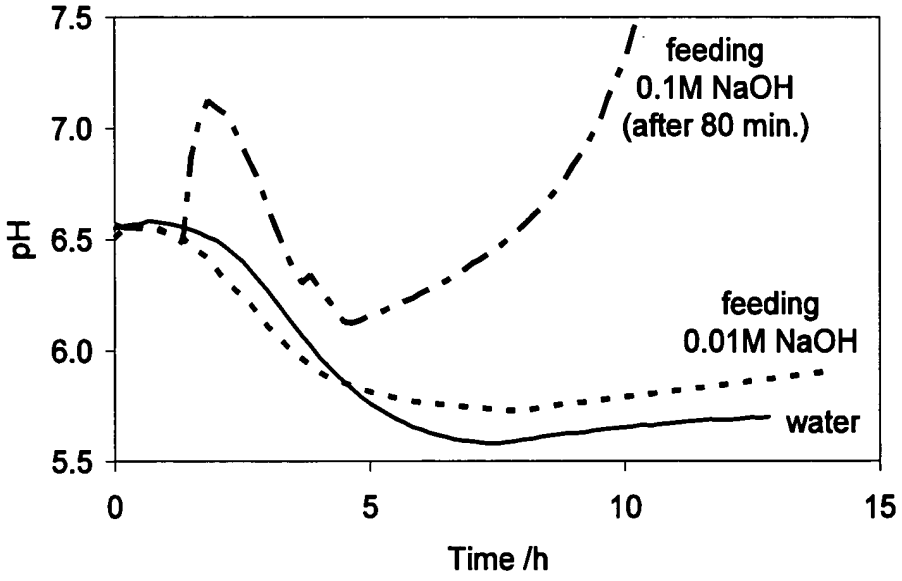


Figure 47A

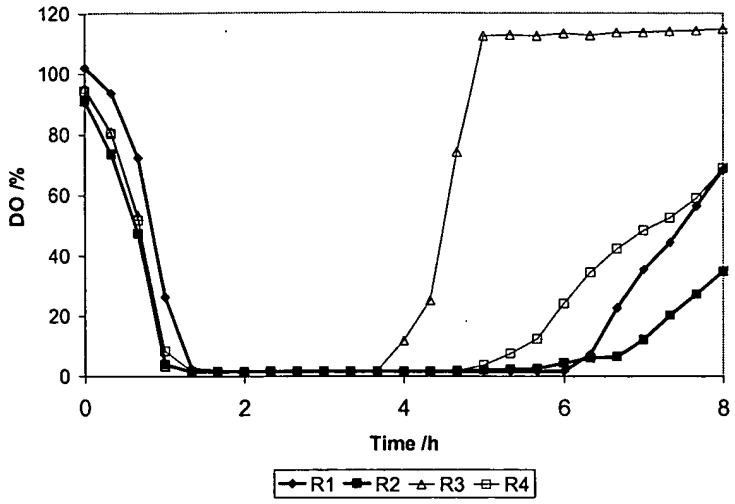


Figure 47B

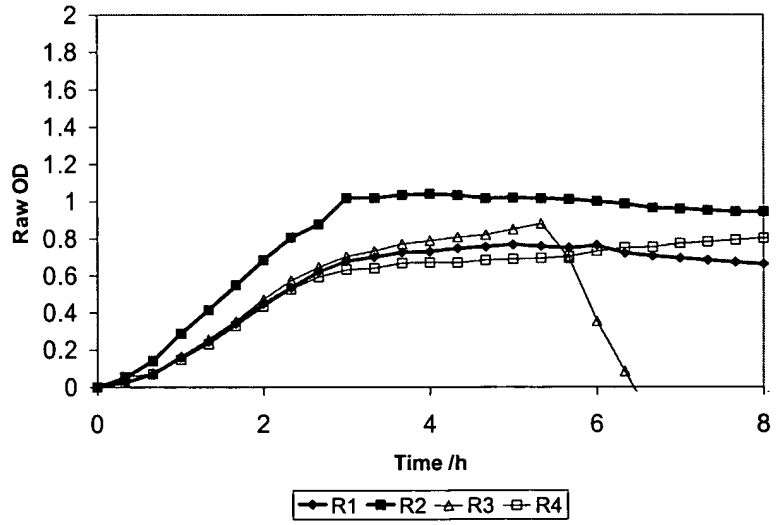


Figure 47C

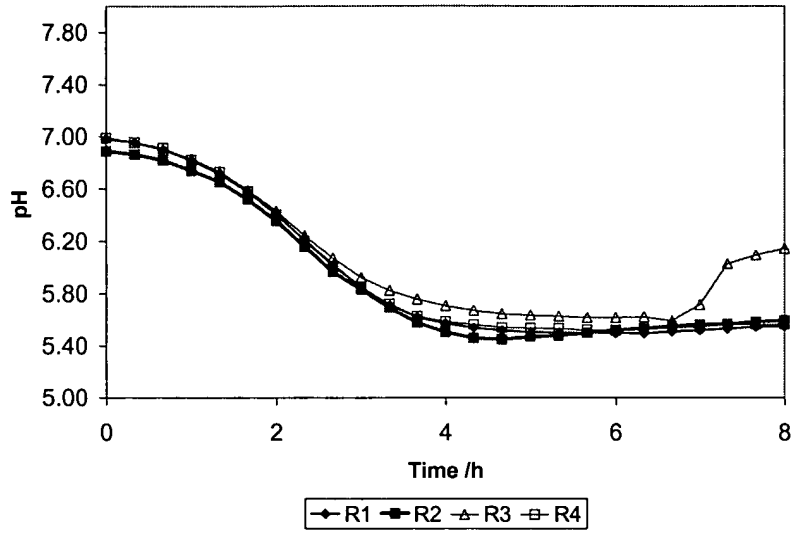


Figure 48A

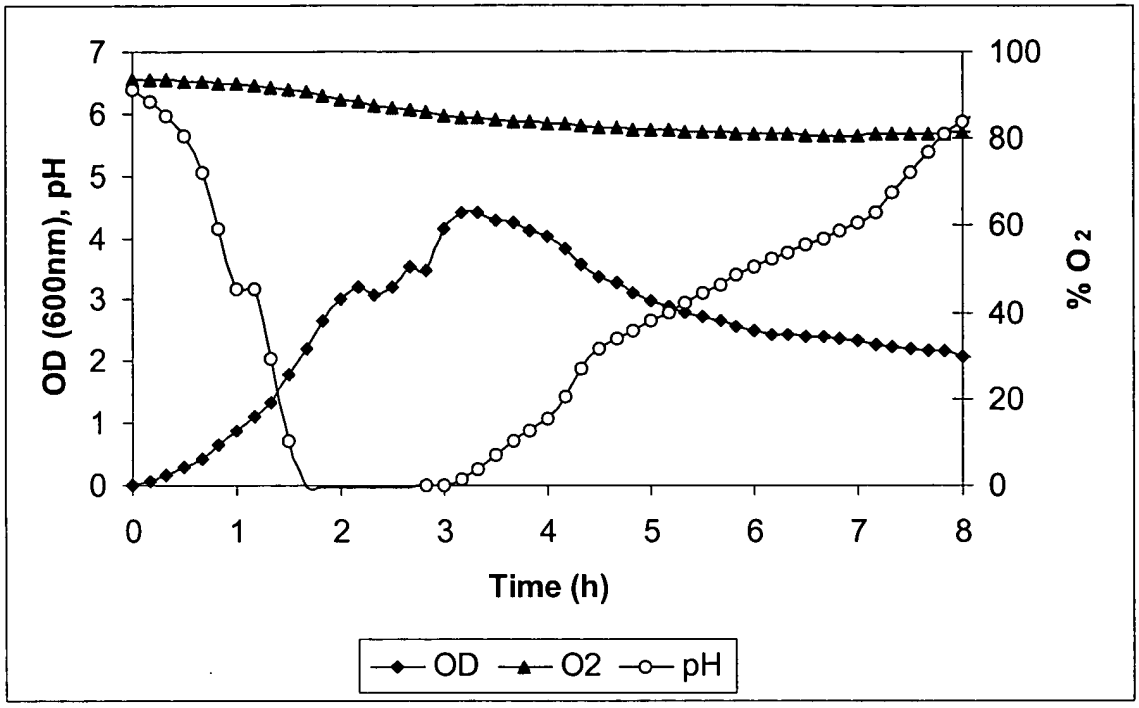
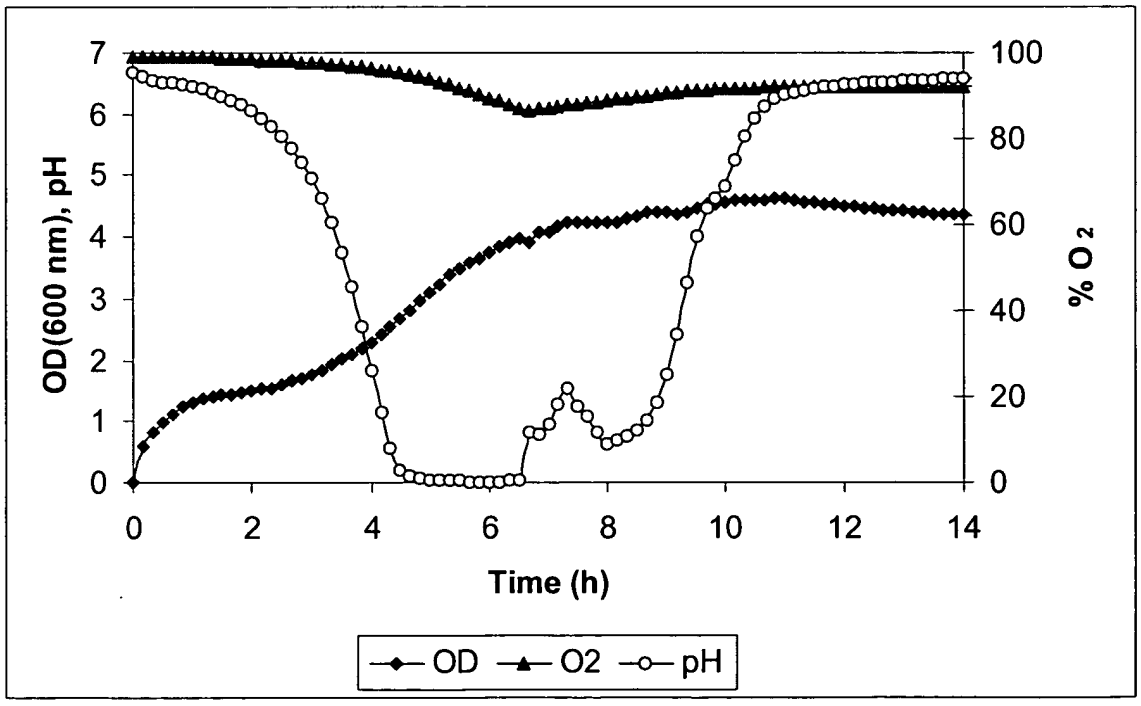



Figure 48B